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VOLUME XII  
1913

CHICAGO  
AMERICAN MEDICAL ASSOCIATION  
PUBLISHERS

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# The Archives of Internal Medicine

Vol. XII

JULY, 1913

No. 1

## THE PULSE FLOW IN THE BRACHIAL ARTERY \*

### I. TECHNIC AND GENERAL CONSIDERATIONS

A. W. HEWLETT, M.D., AND J. G. VAN ZWALUWENBURG, M.D.  
ANN ARBOR

#### DEFINITION OF THE PULSE FLOW

In determining the blood-flow to the arm by a method which we have described,<sup>1</sup> the arm is enclosed in a plethysmograph and the venous out-flow is obstructed by suddenly raising the pressure in a cuff placed just outside of the instrument. The pressure applied is not sufficiently high to interfere with the free entrance of arterial blood. As a result of this obstruction, the arm begins to swell. The rate of swelling is at first fairly uniform; but later, as the pressure in the blood-vessels rises, there is a falling off in the rate of swelling due, on the one hand, to an escape of venous blood beneath the pressure cuff, and on the other to the force opposed to the entering arterial stream by the rising pressure in the capillaries and arterioles. For a brief period, however, the inflow corresponds to the normal rate of blood-flow to the part.

Tracings of the arm volume obtained by this method always show a series of pulse waves due to the intermittent entrance of blood through the arteries; and just as the average rate of arm swelling indicates the average rate of arterial inflow, so the variations on the tracings occasioned by the arterial pulse indicate the variations of inflow due to each portion of the individual pulse waves. Such tracings may therefore be used to determine how much blood is entering the plethysmograph during each portion of the pulse cycle. Since the point at which the blood enters is at the upper end of the plethysmograph, just above the elbow, and since the main path of arterial inflow at this point is through the brachial artery, the records indicate chiefly the rate of blood-flow at this level of the brachial artery during each portion of the pulse cycle. This may be designated the pulse flow in the brachial artery.

\*From the Department of Internal Medicine, University of Michigan.

\*Submitted for publication, March 20, 1913.

1. Hewlett, A. W., and Van Zwaluwenburg, J. G.: The rate of blood flow in the arm. *Heart*, 1909, i, 87.

## TECHNIC

As in our method for determining the blood-flow to the arm, the latter was enclosed in an air plethysmograph which was connected by air transmission with an apparatus which recorded the changes in arm volume. In order to obtain records of the average blood-flow to the arm, any form of volume recorder may be used. We early used the bellows volume recorder of Brodie for this purpose, but more recently we have used a bell recorder similar to that employed for recording respiratory movements. To obtain accurate records of the flow during each portion of the pulse cycle, however, a more sensitive recorder becomes necessary. In a series of exhaustive researches, O. Frank<sup>2</sup> has discussed the necessary requisites of a recorder which shall follow sudden changes in movement with accuracy. The most important of these requisites is that the apparatus itself should possess a rapid rate of inherent vibration. A slow rate of inherent vibration indicates that the apparatus will not follow sudden changes in movement accurately and that the vibrations of the apparatus markedly deform the tracings. With a rate of inherent vibration which is very rapid relative to the changes in movement to be recorded, the latter are recorded much more accurately, and the vibrations of the instrument can be easily recognized should they appear on the tracings.

We have, therefore, endeavored to employ an apparatus which should have as rapid an oscillation time as possible. The following formula furnishes an approximate idea of the factors affecting the time of a single oscillation,  $T$ , in a system similar to that which we have employed.

$$T = 2\pi \sqrt{\frac{\frac{L}{A} d + M}{\left(\frac{1}{V} + E\right) C}}$$

In this formula,  $A$  and  $L$  represent, respectively, the cross section and the length of the tube connecting plethysmograph and recorder, and  $d$  is the specific gravity of the enclosed air.  $M$ , which represents the moment of inertia of the recorder, depends on its weight, and the square of the distance through which it is moved.  $V$  is the volume of air in the plethysmograph, and  $E$  is the rise of pressure produced when one unit of air enters the recording device. The factor  $C$  is a constant depending on the atmospheric pressure and the adiabatic correction for air. The above formula is constructed on the assumption that com-

2. Frank, O.: Kritik der elastischen Manometer. *Ztschr. f. Biol.*, 1903, xliv, 445, and a series of articles in the same journal.

pression of air in the transmission tube may be disregarded and that no damping factor is present.<sup>3</sup>

Inspection of the above formula shows that in order to lessen the time of a single vibration in the apparatus the length of the connecting tube, the weight and movement of the recording device and the volume of air in the plethysmograph should be as small as possible; while the cross section of the tube should be as large as possible. In shortening the tube we have endeavored to leave a length which will be sufficient to allow manipulations about the plethysmograph and to allow the individual to move his arm in the suspended plethysmograph without putting traction on the rigidly fixed recorder. For this purpose a length of about 60 cm. is necessary. The connecting tube used had a diameter of 1.2 cm. Between 2,000 and 2,700 c.c. of air usually occupied the portion of plethysmograph about the arm. By far the most important part of the apparatus, however, is the recording device. Two types of sensitive recorders were experimented with. In one of these a soap bubble was placed over the end of the tube leading from the plethysmograph, and the oscillations of its summit occasioned by the volume changes in the arm were photographed on a moving sensitized surface. Garten<sup>4</sup> has published plethysmograph tracings taken by this method and we have attempted to use it for recording the pulse flow in the brachial. After a rather extended trial of the method, however, we became convinced that the oscillation time of the apparatus was inadequate. When connected with a tube sufficiently long to permit fixation of the carrier of the soap bubble the oscillation time was usually between 9 and 13 per second, which was slow for our purposes. Garten's tracings also suggest that the oscillation time in his apparatus was slow, as has been pointed out by O. Frank. The reason for this slow time even when the recorder itself is of negligible weight, becomes evident on inspection of the above formula. In the soap bubble which is essentially a volume recorder the rise of pressure when one unit of air is added to the recorder is practically nil.  $E$ , therefore, approaches zero, and since the fraction  $1/V$  is also very small, the period of oscillation is relatively long. We were able to get very satisfactory plethysmograph tracings of the finger by this method, for in this case the volume of air surrounding the finger is small and consequently the fraction  $1/V$  is relatively large. A trial soon convinced us, however, that our method for determining the blood-flow could not be applied satisfactorily to the finger.

3. In the system finally adopted the tube was relatively large and only a very small amount of air entered the recorder. Under such circumstances the compression of air in the tube becomes an important factor which cannot be disregarded. The more complex formula for such a system need not be discussed here.

4. Garten, S.: Ueber ein neues Verfahren zur Verzeichnung von Bewegungsvorgängen und seine Anwendung auf den Volumenpuls. *Arch. f. Physiol.*, 1904, civ, 351.

Since a true volume recorder, even though of negligible weight, necessarily gives slow oscillations when connected with an arm plethysmograph, it became necessary to adopt some other principle for recording the volume changes in the arm. The principle which we adopted was to close the end of the plethysmograph about the arm with unyielding material and to use a Frank capsule as a recorder. The latter is essentially a small Marey tambour in which the recording lever has been replaced by a small mirror which directs an imponderable beam of light on a moving sensitized surface. A description of the apparatus used has been published elsewhere.<sup>5</sup> Since but a very small amount of movement occurs in the Frank capsule during changes in arm volume, the system becomes a pressure system rather than a volume system, and the changes in pressure are recorded by the capsule. By calculating the air in the plethysmograph after the arm is in place, it is possible, however, to graduate the capsule against an equal volume of air in a bottle by allowing measured amounts of water to enter the bottle. The records can thus be read as volume records. With this apparatus high rates of vibration may be obtained. In the tracing shown in Figure 1, for example, the oscillatory frequency of the apparatus was over 100 per second. Some of the tracings in the following articles were taken with an apparatus of much slower vibration time, 15 to 20 per second, but our main conclusions as to the features of the pulse form have been checked by tracings taken with the more sensitive apparatus.

The steps followed in making a series of records were as follows: The volume of arm to be enclosed in the plethysmograph was determined by thrusting it into a large cylinder full of water and measuring the amount of water displaced. The arm was then placed in the plethysmograph, and the primary closure of the end was made by rubber dam. In order to make this closure a rigid one the rubber dam was reinforced by a layer of dentist composition which had been previously softened in hot water. This was applied closely about the arm and over the end of the plethysmograph. While the composition was hardening a large bottle was filled with water until the contained volume of air was equal to the air remaining in the plethysmograph after insertion of the arm. The bottle was then connected with the Frank capsule and the latter was graduated by allowing successive amounts of water, 2 c.c. each, to enter the bottle. The vibration time of the apparatus may then be tested by suddenly adding a small amount of air to the bottle and photographing the after-oscillations of the tambour. The Frank capsule was then connected with the plethysmograph and the apparatus was ready for making records. These latter were made in the same manner as our

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5. Van Zwaluwenburg, J. G., and Agnew, J. H.: Some Details of the Auricular Pressure Curves of the Dog. *Heart*, 1912, iii, 343.



usual records of the blood-flow, except for the form of recorder used. It was found convenient to have an additional tube leading from the plethysmograph in order to regulate the position of the light. This additional tube was left open at the moment that the pressure was applied to the arm, but was closed immediately after. In this way the disturbance that is frequently produced at the moment of applying the pressure was not recorded. Each record taken by the Frank capsule was furthermore checked as to the average blood-flow by a record taken by the usual volume recorder on a moving drum covered by smoked paper.

#### FACTORS INFLUENCING THE PULSE FLOW

Following Chauveau and Marey, French physiologists usually resolve the blood-flow in an artery into two chief components, the constant and the intermittent flows. Somewhat similarly we have also found it convenient to analyze the pulse flow into two components. The first of these is the average rate of blood-flow in the arm as determined by our ordinary method. The second is the intermittent acceleration or retardation of this flow occasioned by the progress of fluid waves in the artery. These latter are recorded in good plethysmograph tracings if one assumes that the venous outflow does not change materially with each portion of the arterial pulse. Knowing the average rate of blood-flow to an organ it is possible to construct the pulse flow from a good quantitative plethysmographic tracing by erecting on the slant which represents the average flow, verticals for each portion of the plethysmograph curve and connecting their summits. A construction of this type is shown in Figure 1, C. Its identity with the curve obtained directly (Fig. 1, A) is at once apparent.

The velocity in cubic centimeters per unit of time for each portion of the pulse cycle can also be constructed from our pulse flow curves; for the velocity at any moment is the amount of inflow divided by the time. This is represented on each portion of the pulse cycle by the trigonometric tangent to the curve at this point. Constructions of this sort by the method described by Fick<sup>6</sup> are shown in Figure 1, D, and Figure 2, B. These are absolute velocity curves or absolute tachograms in terms of volume flow. They differ from the velocity curves constructed by Fick from plethysmograph tracings and from the tachograms obtained directly by V. Kries,<sup>7</sup> in that they represent absolute volume velocities in the brachial artery and not simply changes in velocity which are superimposed on an unknown constant. If one knew the exact diameter of the brachial artery these volume velocities might be converted into linear velocities. The close resemblance of our volume velocity constructions

6. Fick: *Gesammelte Schriften*, Würzburg, 1904, iii, 550.

7. Kries, V.: *Studien zur Pulslehre*. Freiburg, 1892.

to the variations in linear velocity determined directly on animals may be seen by comparing our construction with the velocity curves frequently published in standard works in physiology.<sup>8</sup> We see no advantage, however, in converting our pulse flows into pulse velocities.

Not only may the pulse flow be resolved into the two components, average flow and wave motion, but the two appear to us to depend on factors which are more or less independent of one another. The average rate of blood-flow in an artery depends in the main on the average arterial pressure and the amount of resistance offered to the flow of blood through the arterioles and capillaries supplied by the artery under consideration. A limited area of vasodilation will greatly accelerate the local blood-flow although it may have but little effect on the general level of arterial pressure. The size and form of the pulse waves as distinguished from the constant flow may, theoretically at least, vary widely even though the flow remains constant. The pulse waves depend primarily on the systolic output from the heart and it is to be expected that they will vary with variations in the amount of blood expelled from the heart and with the character of the expulsion, rapid, slow, etc. But the volume of the pulse entering any particular arterial trunk is also dependent on the relative size of this trunk. If for example the main artery leading to a part widens and the conditions throughout other parts of the circulatory apparatus remain unchanged, a more voluminous wave will enter this artery even though the size of the arterioles and capillaries which drain it remain unchanged. The pulse waves are also greatly modified by their passage through the arterial tree. Changes in the elasticity of the arterial walls, the tendency of the arteries to impart their own oscillation times to waves coming to them, the reflection of waves at the periphery, all these render the finer analysis of the peripheral pulse a matter of great complexity.

Thus far we have not used the above method on animals. Nor can it be confidently predicted to what degree our results on the arm of man can be transferred to the pulse flows in other organs or to the flow in the extremities of other animals. It must be remembered that the pulse flow in each organ is dependent more or less on local vascular conditions. Variations in the size of the main artery leading to the part, in the elasticity of this artery, in its length and distance from the heart, and in the condition of the finer arterioles and capillaries will necessarily modify the picture of the pulse flow. We know that in the arm of man the blood-flow usually maintained is far short of that which is possible under special conditions, such as local muscular exercise or the local or general application of heat. So far as we know, no studies of the absolute pulse flow in an artery as distinguished from the linear velocity had appeared

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8. Marey, E. J.: *La circulation du sang*, Paris, 1881, Fig. 188.

previous to our preliminary report.<sup>9</sup> Since that time, Hürthle<sup>10</sup> has recorded the pulse flows in the arteries of the dog by means of a specially constructed *Stromuhr*. His tracings obtained from the carotid and the femoral arteries differ considerably from those which we have obtained from the arm of man, especially in regard to the finer waves present. The differences between our tracings and those of Hürthle are doubtlessly due in part to the difference in the arteries selected for study. Aside from this difference in the material used, however, it is evident from Hürthle's statements that his instrument was far less sensitive than was ours to rapid variations in the blood-flow, and in particular he states that on account of its lack of sensitiveness reflected waves may escape record by the *Stromuhr* which he used. From our studies we believe that reflected pulse waves are of great importance in certain types of the brachial pulse flow in man and that any method which fails to record these accurately misses one of the most interesting and important features of the pulse flow.

In the following articles certain types of marked modification of the pulse flow in the brachial artery will be presented, and we shall attempt to explain the causes of these modifications in accordance with the above considerations, so far as this seems possible. It is not to be expected that our explanations will be free of error, but we hope, at least, that our objective records will throw light on some of the complex problems of the peripheral arterial pulse of man.

## II. RELATION TO THE AVERAGE BLOOD-FLOW. EFFECT OF NITROGLYCERIN

A. W. HEWLETT, J. G. VAN ZWALUWENBURG AND J. H. AGNEW  
ANN ARBOR

### GENERAL FORM OF THE PULSE FLOW

As a rule the pulse flow in the brachial artery follows a fairly uniform course. There is a sudden and marked increase in the flow when the main pulse wave arrives from the heart (Figs. 1 and 3). Immediately following this primary wave there is a momentary slowing in the flow, which sometimes becomes a momentary backward movement of the blood in the artery. This is interrupted by the positive dicrotic wave, after which there is, as a rule, comparatively little movement in the column of blood in the artery up to the entrance of the next primary wave from the heart. What little movement takes place during diastole is usually in

9. Hewlett, A. W.: Van Zwaluwenburg, J. G., and Agnew, J. H.: A New Method for Studying the Brachial Pulse of Man. *Tr. Assn. Am. Phys.*, 1912, xxvii, 188. Read May 14, 1912.

10. Hürthle, K.: Ueber die Beziehung zwischen Druck und Geschwindigkeit des Blutes in den Arterien. *Arch. f. Physiol.*, 1912, cxlvii, 525.

the positive direction, although it seems possible to have a stationary column of blood or even a slight continuous backward movement toward the heart during diastole without pathological significance. In addition to the main waves described above, there are frequently finer oscillations on the tracings other than those occasioned by movements on the part of the patient.

RELATION BETWEEN THE PRIMARY PULSE WAVE AND THE  
BLOOD-FLOW

It has been pointed out in the preceding article that the pulse flow may be resolved into two components, the average blood-flow to the part and the variation in this flow produced by the arterial pulse waves. Of these waves the primary pulse wave is the largest and most important, and one of the first questions that arises is whether any relation exists between the size of the primary wave and the average rate of blood-flow to the arm. May one, in other words, draw any conclusion as to the rate of blood-flow in the arm from the size of the volume pulse? This question has been tested in two ways: first, by comparing the blood-flows and the volume pulses of different individuals under fairly uniform conditions, and second, by comparing them in a single individual under varying conditions. In the following table we have recorded the average rates of blood-flow in a number of individuals together with the heights of the primary pulse waves, as measured above the average blood-flow.

TABLE 1.—RELATION BETWEEN HEIGHT OF PRIMARY WAVE AND RATE OF  
BLOOD-FLOW IN DIFFERENT INDIVIDUALS

Name	Diagnosis	Height of primary waves in c.c.	Total amount of blood entering the arm in a complete pulse cycle in c.c.	Average blood- flow in c.c. per 100 c.c. of arm substance per minute
Bis.....	Patent ductus arteriosus .....	0.66	0.4	1.7
Cr.....	Neurasthenia .....	0.6	0.5	1.8
Fli.....	Rheumatism .....	0.4	0.4	2.3
Eas.....	Arthritis .....	0.9	0.7	2.6
McQu.....	Aneurism .....	0.5	0.5	3.2
Smi.....	Pleurisy .....	0.5	0.7	3.6
Gui.....	Diabetes and arteriosclerosis .....	0.95	0.7	3.8
Hic.....	Hypertension .....	0.6	0.8	4.5
Mus.....	Hypertension .....	0.6	1.2	4.6
Haz.....	Hypertension .....	0.6	0.9	5.4
Cha.....	Pernicious anemia ....	0.45	0.8	5.7
Mor.....	Pernicious anemia ....	0.6	1.5	6.0
Fly.....	Hypertension .....	0.9	1.1	6.1
Stem.....	Hypertension .....	0.7	1.0	6.2

It is evident from this table that in different individuals the size of the volume pulse gives no indication of the rate of blood-flow to the arm.



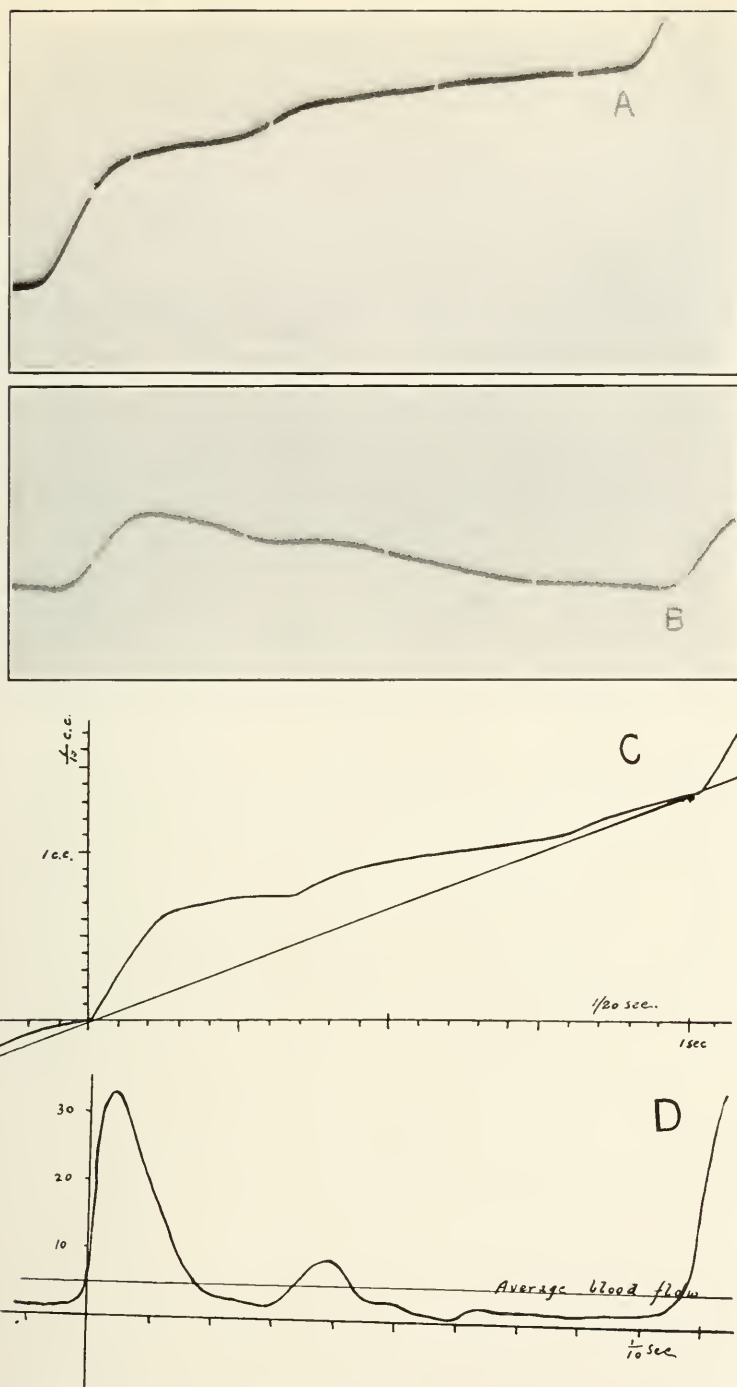


Fig. 1.—The relation between pulse flow, plethysmogram, and tachogram. Instrumental oscillations 106 per second. A is the pulse flow tracing. B is an ordinary plethysmogram taken from the same individual when the venous out-flow from the arm was not obstructed. C is a construction of the pulse flow from the plethysmogram and from the average flow of blood as determined by our usual method. D is a velocity construction or tachogram. This was constructed from A by erecting trigonometric tangents of the curve on an abscissa representing the time. In all of our figures the horizontal interruptions of the tracings represent differences of volume of 2 c.c. and the vertical interruptions are produced by a time marker. Two vertical interruptions represent one-half second. The average rate of blood-flow was 5 c.c. per 100 c.c. of arm substance per minute. The primary wave put in 0.46 c.c. above the average rate of flow. The total amount of blood entering the arm with one complete pulse cycle was 1.35 c.c.

The blood flow to the arm may be markedly influenced by the room temperature<sup>11</sup> and by exercise of the arm within the plethysmograph.<sup>12</sup> In one set of experiments a single individual was subjected to a variety of room temperatures. When the room temperature was agreeable the blood-flow to the arm usually ranged between 3 c.c. and 6 c.c. per 100 c.c. of arm substance per minute; during marked chilliness it fell to 1.5 c.c., and during uncomfortable warmth it rose to 13.7 c.c. During agreeable room temperatures the flow pursued the course already outlined. The

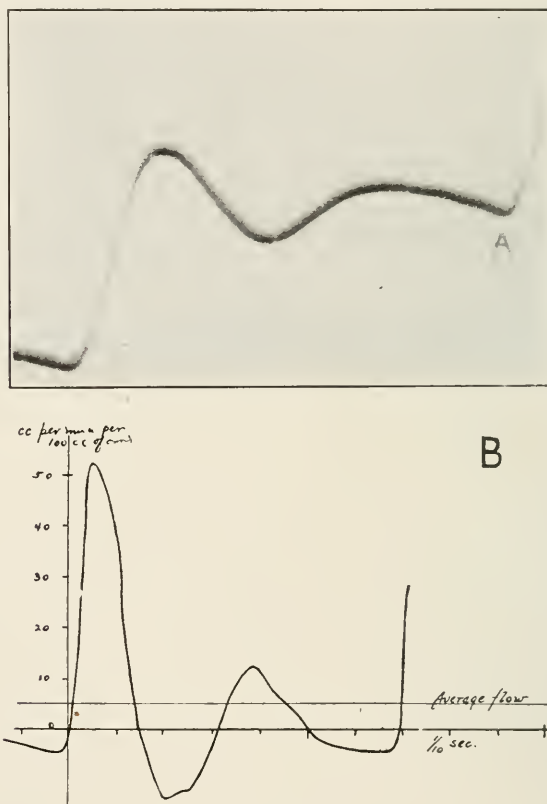


Fig. 2.—The pulse flow and the constructed tachogram in a typical nitroglycerin pulse. From the same individual as Figure 1. Instrumental oscillations 106 per minute. Note the increase in the size of the primary wave, the marked negative wave immediately following, and the elimination of smaller secondary waves. Average rate of blood-flow 5 c.c. per 100 c.c. of arm substance per minute. The primary wave put in 1.3 c.c. above the average rate of flow. The total amount of blood entering the arm in a complete pulse cycle was 1.1 c.c.

11. Hewlett, A. W.: The Effect of Room Temperature upon the Blood Flow in the Arm, with a few Observations on the Effect of Fever. *Heart*, 1911, ii, 230.

12. Hewlett, A. W., and Van Zwaluwenburg, J. G.: The Rate of Blood Flow in the Arm. *Heart*, 1909, i, 87.

primary wave delivered from 0.45 to 0.9 c.c. of blood measured above the average rate of inflow, and there was comparatively little movement in the column of blood from the end of one primary wave to the beginning of the next. When the individual was chilled and the blood-flow to the arm was reduced to a minimum, there was usually a corresponding reduction in the size of the primary pulse wave so that the main inflow to the arm still occurred during the early part of systole, and from then on the flow practically ceased. There was, however, a tendency for the reduction in flow to be somewhat greater than the reduction in the primary wave, which resulted, in some tracings, in a tendency to a slight back-flow of blood between the primary pulse waves.

When the room was heated and the flow of blood was markedly increased, there was but a moderate increase in the height of the primary pulse wave as measured above the average rate of blood-flow. The flow

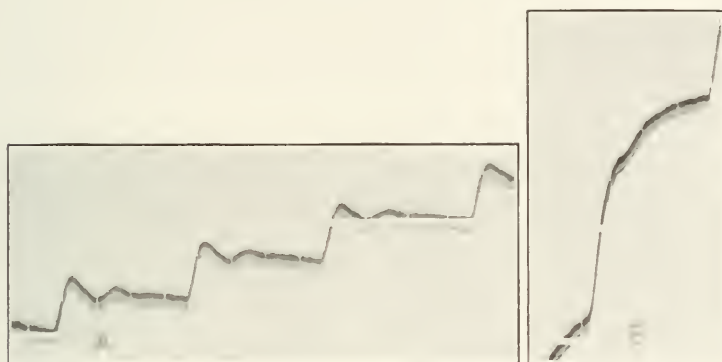


Fig. 3.—The effect of a marked change in the blood-flow on the pulse flow. Instrumental oscillations 46 per second. A. The ordinary flow was 2.4 c.c. per 100 c.c. of arm substance per minute. The primary wave was large relative to this flow. A part of the primary wave was immediately reflected during the systolic portion of the pulse and the tracing shows a slow backward flow during diastole. B. Following exercise of the arm within the plethysmograph the flow was increased to about 20 c.c. per 100 c.c. of arm substance per minute. The flow in the brachial artery more nearly approaches a straight line.

between primary waves was, however, markedly increased. Instead of the step-like entrance of blood into the arm which characterizes the usual flow into the brachial artery, there was now more of a continuous flow throughout all parts of the pulse cycle. This same type of tracing was also obtained in the rapid flow following exercise of the muscles within the plethysmograph (Fig. 3), and it was occasionally encountered in patients with unusually rapid rates of flow. The explanation of this change in the form of the pulse flow appears obvious. Ordinarily the amount of blood delivered to the arm by the primary pulse wave is about equal to that which is allowed to escape through the finer arterioles

during the entire pulse cycle. When, however, a very rapid flow of blood through the part is demanded, the arterioles relax and the large amount of blood passing through them can no longer be supplied by the primary pulse waves. Consequently the flow in the brachial artery becomes more or less continuous.

It is apparent, therefore, that even in a single individual the size of the volume pulse is no accurate guide to the rate of blood-flow through the arm. This is especially true of very rapid flows, for in these the rate of flow is always increased to a far greater extent than is the size of the primary waves. We shall return to this lack of parallels between the blood-flow and the size of the primary waves in the discussion of the nitroglycerin pulse, where very large primary waves are associated with relatively slow flows (Table 2).

TABLE 2.—RELATION BETWEEN HEIGHT OF PRIMARY WAVE AND RATE OF BLOOD-FLOW IN A SINGLE INDIVIDUAL WITH AND WITHOUT NITROGLYCERIN

Without Nitroglycerin			With Nitroglycerin		
Height of Primary Wave in c.c.	Total Blood Entering in One Pulse Cycle in c.c.	Average Flow in c.c. Per 100 c.c. of Arm Per Minute	Height of Primary Wave in c.c.	Total Blood Entering in One Pulse Cycle in c.c.	Average Flow in c.c. Per 100 c.c. of Arm Per Minute
0.36	0.41	1.5	0.86	0.33	1.5
0.26	0.50	2.0	1.09	0.35	1.7
0.77	0.72	3.1	1.10	0.52	2.1
0.96	1.47	6.3	1.06	0.99	4.6
0.95	1.48	6.9	0.95	1.14	5.4
0.90	2.5	13.7	1.20	1.34	6.0
			1.12	1.53	7.1

#### NEGATIVE OR BACK-FLOWS IN THE BRACHIAL ARTERY

Indications of backward movement of the blood-column in the brachial artery are frequently encountered in our tracings. These backward movements appear to be of two varieties. In the first the flow is of short duration and of wave-like character. This type of negative flow is most apt to occur during the late systolic period of the pulse, immediately after the primary wave. As it is particularly well marked in the nitroglycerin pulse its discussion will be deferred until that pulse is considered.<sup>13</sup>

The other type of backward movement is of a more continuous character and extends from the diastolic wave to the beginning of the next primary wave. This continuous, diastolic backward flow is particularly

13. For convenience we shall speak of the systolic period of the pulse as the portion preceding the beginning of the diastolic wave, although we are aware that this may not exactly represent the period of ventricular systole.

marked in many tracings from aortic insufficiency, but it is occasionally indicated even in normal individuals (Fig. 3). Although tracings from normal individuals occasionally show this continuous diastolic back-flow, the accuracy of these tracings might be questioned. The retrograde movement on the tracings is never very marked and should our records underestimate the true blood-flow by 10 or 20 per cent., then the supposed negative flow would usually disappear. We are inclined to believe, however, that a slight continuous diastolic back-flow may occasionally occur in the brachial artery, even in normal individuals. Theoretically, at least, this seems possible. The flow between the primary pulse waves depends on the relation which exists between the amount of blood put into the brachial artery by the primary wave and the amount escaping through the arterioles during the entire pulse cycle. The latter is proportional to the average blood-flow. If the average flow is fast relative to the size of the primary waves then the flow between beats will be positive and rapid, as is seen after local exercise (Fig. 3). If, on the other hand, the average flow is relatively slow, then the flow between beats may be reduced to zero or even to a negative quantity (Fig. 3). These two factors, average flow and size of primary waves, are certainly more or less independent of each another. The former depends in the main on the blood-pressure and the resistance encountered in the smaller arterioles and capillaries. The latter depends on various factors, especially the amount of ventricular systole and the size of the local arteries. If, for example, the finer arterioles become constricted so as to cut down the average blood-flow and at the same time the systolic output from the heart and the size of the larger arteries leading to and in the arm are not affected, then a large pulse wave will enter the brachial artery. Not all of this blood can escape into the capillaries and veins during the pulse period, and some will, therefore, be returned toward the aorta between pulse beats. Apparently this return may be in the form of a sudden wave late in systole or as a more continuous flow during diastole or as a combination of the two. Such a return of blood from the brachial artery need cause no rise in the general arterial pressure, for the flow through other organs may be so free as to counterbalance the local resistance encountered in the arm.

#### THE NITROGLYCERIN PULSE

Nitroglycerin, even in such small doses as one drop of the 1 per cent. alcoholic solution placed on the tongue, usually produced marked changes in the form of the peripheral pulse. These changes begin in two or three minutes, reach their maximum in five or six minutes, and pass off in fifteen minutes or more. The radial pulse becomes larger and fuller, and the pulsations recorded by the Erlanger sphygmomanometer become



larger.<sup>14</sup> Plethysmograph records of the arm usually show arm swellings of 15 c.c. or more, amounts which are of the same order of magnitude as those produced by the brief applications of heat or cold to distant parts of the skin.<sup>15</sup> The plethysmograph pulse becomes distinctly larger. Changes in the blood-flow to the arm also occur, but we have found the flows to be sometimes faster and sometimes slower, and on the whole, they are rather difficult to follow. The marked increase in blood-flow which might be anticipated from the arm swelling and from the increase in the size of the volume pulse is not realized.

This lack of parallelism between the arm swelling and the local blood-flow is noteworthy, because it is often assumed that a rapid increase in the size of an organ is trustworthy evidence of an increase in the local blood-flow. Such a parallelism seems to exist when the arm is subjected to thermic influences,<sup>16</sup> but it is certainly absent in nitroglycerin action. This disproportion between arm swelling and blood-flow may occur even when the systolic blood-pressure is but slightly reduced, and we have attributed it to a dilatation of the arterial trunks without a corresponding relaxation of the arterioles which are believed to govern the local flow. The somewhat similar observation of Bunch<sup>17</sup> that the submaxillary gland may decrease in size even though the local blood-flow increases, has been held to be due to a reduction in the size of the gland by loss of secretion.

The changes in the brachial pulse flow produced by nitroglycerin are so characteristic, that in going over a set of records, the nitroglycerin tracings can usually be recognized with ease. Their main features, readily seen by comparing Figure 2, A with Figure 1, A, are the large size of the primary wave, the marked negative wave immediately following, the large dicrotic wave, and the elimination of other secondary oscillations. In the individual particularly studied, the primary pulse waves at the height of the drug action were always very large, equalling or exceeding the primary waves which accompanied the most rapid blood-flows caused by a warm room. (See Table 2.) Even when the flow of blood through the arm was greatly slowed by a chilly room, the primary waves of the nitroglycerin pulse remained large.

Two explanations of the large nitroglycerin pulse seem possible. On the one hand it is conceivable that this might depend on the manner in

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14. Hewlett, A. W.: The Effect of Amyl Nitrate Inhalations upon the Blood-Pressure in Man. *Jour. Med. Research*, 1906, xv, 383.

15. Müller, O.: Ueber die Blutverteilung im menschlichen Körper unter dem Einfluss thermischer Reize. *Deutsch. Arch. f. klin. Med.*, 1905, lxxxii, 547.

16. Hewlett, A. W., Van Zwaluwenburg, J. G., and Marshall, Mark: The Effect of Some Hydrotherapeutic Procedures on the Blood-Flow in the Arm. *THE ARCHIVES INT. MED.*, 1911, viii, 591.

17. Bunch, I. L.: On the Changes in Volume of the Submaxillary Gland During Activity. *Jour. Physiol.*, 1900, xxvi, 1.

which the blood is expelled from the heart. A large systolic output or a sudden emptying of the ventricle against a low arterial pressure might cause a pulse wave of unusual height, which was propagated as such into the brachial artery. On the other hand, the large pulse wave might be due to a relaxed condition of the larger vessels in the arm. Animal experiments<sup>18</sup> have shown that nitroglycerin increases the systolic output in some dogs, but not in others. The effect on the cardiac output in man is not known, for conclusions drawn from changes in pulse pressure are open to numerous criticisms.<sup>19</sup> On the other hand, there is positive evidence in favor of the view that the nitroglycerin changes in the brachial pulse of man are due to peripheral influences, for we have repeatedly found that carotid tracings taken by tambours do not show these changes even when the brachial tracings do (Fig. 4).

Carotid tracings are fairly representative of the central aortic pulse, and we are, therefore, inclined to attribute the characteristic changes

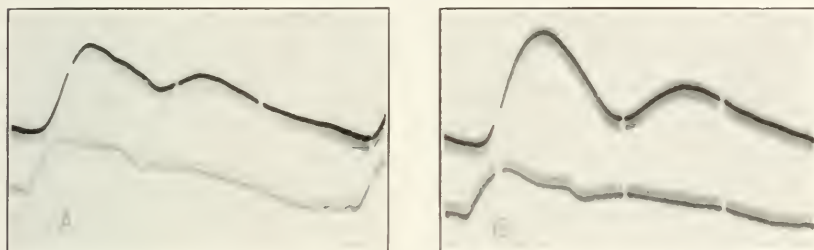


Fig. 4.—Pressure tracings from the carotid artery (above) and from the brachial artery (below), before (A) and after (B) the administration of nitroglycerin. Note that the brachial was affected to a far greater extent than the carotid.

of the nitroglycerin pulse to changes in the peripheral arteries. A relaxed condition of the arterial trunks leading to and distributed in the arm would permit a large undamped wave to enter the brachial artery. Direct evidence in favor of the arterial relaxation is found in the obliterations of the smaller arterial oscillations, for Veiel<sup>20</sup> and Müller and Weiss<sup>21</sup> have shown that these tend to disappear from the pressure pulse of the radial artery when the tonus of the larger arterial trunks is diminished.

18. MacNider, W. de B.: The Action of the Nitrites on the Heart. *Am. Jour. Med. Sc.*, 1908, cxxxv, 99.

19. Müller, O.: Der arterielle Blutdruck und seine Messung beim Menschen. *Ergebn. d. inn. Med. u. Kinderh.*, 1908, ii, 367.

20. Veiel, E.: Ueber die Bedeutung der Pulsform. *Deutsch. Arch. f. klin. Med.*, 1912, cv, 250.

21. Müller, O., and Weiss, E.: Ueber die Topographie, die Entstehung und die Bedeutung des menschlichen Sphygmogrammes. *Deutsch. Arch. f. klin. Med.*, 1912, cv, 320.

## PERIPHERAL REFLECTION OF THE PRIMARY PULSE WAVE

The brief negative wave of late systole which sometimes follows the primary wave even on normal tracings is very markedly accentuated in the nitroglycerin pulse. There seems to be no possibility of attributing this negative wave to an experimental error, for it is much too prolonged to be due to a defect in the recording apparatus, and the negative flow is far too rapid to be explained as due to any error in the estimation of the average blood-flow. To our minds no explanation for this negative wave seems possible, other than that it is due to a momentary and marked wave of back-flow in the brachial artery. Although the velocity tracings that have been taken directly from animals are open to the criticism, that the instruments used were not especially adapted for recording sudden changes in movement, nevertheless, it is interesting to note that in some of these<sup>22</sup> a negative velocity has been recorded just following the marked positive velocity produced by the primary pulse wave. Furthermore, in v. Kries' tachograms, as well as in one published by O. Frank,<sup>23</sup> the greatest reduction in velocity occurred just after the main wave, and v. Kries<sup>7</sup> has shown that this reduction of velocity is particularly well marked in certain stages of amyl nitrite action. These records of absolute and of relative pulse velocities, therefore, agree with our tracings of the pulse flow in the brachial artery of man in showing that a negative wave in the artery might be expected just following the main pulse wave.

The question arises as to whether this negative wave in the brachial artery is of central or peripheral origin. If of central origin, its explanation would be that frequently given for the dicrotic notch, viz.: a negative wave originating at the beginning of the aorta and produced by the settling back of the blood-column against the semilunar valves. There can be no doubt that a negative wave of this character does originate at the base of the aorta, but it is too small to explain the marked back-flow which occurs in the brachial arteries during the action of nitroglycerin, for O. Frank<sup>24</sup> has shown that the closure of the valves produces only a brief notch in the aortic pressure tracing. The negative flow wave in the brachial artery, on the other hand, may amount to 0.5 c.c., and to over 50 per cent. of the primary wave after giving nitroglycerin. We have, therefore, concluded that in the brachial artery the negative wave of late systole is due in the main to peripheral influences.

There has been considerable discussion among physiologists as to the degree to which peripheral reflection of pulse waves may affect the form of the pulse. It is well recognized that whenever a liquid wave travels along an elastic tube of great length, it will be in part reflected as a

22. Marey, E. J.: *La circulation du sang*, Paris, 1881, Fig. 188.

23. Frank, O.: *Konstruktion und Theorie eines neuen Tachographen*. *Ztschr. f. Biol.*, 1907, 1, 303.

24. Frank, O.: *Der Puls in den Arterien*. *Ztschr. f. Biol.*, 1905, xlv, 441.



positive wave whenever an obstruction is met, and that it will be in part reflected as a negative wave whenever the tube suddenly widens. The resultant of such a combination of reflected positive and negative waves from numerous points in the arterial tree must be extremely complicated, and attempts to explain variations in the pulse form from this point of view have not led to very satisfactory conclusions. O. Frank<sup>24</sup> has, therefore, proposed that the subject be viewed from a somewhat different point of view. He has shown that in the central aortic pulse there is little evidence of the presence of waves reflected from the periphery. On the other hand, the femoral pulse of the dog differs markedly in type from that of the aorta. There is not simply a smoothing out of the general features of the aortic pulse such as one might expect from the passage of a wave along a long elastic tube, but the dicrotic notch is deepened, the pulse pressure is markedly increased and the primary wave may show a higher systolic pressure than actually exists in the aorta. Dawson<sup>25</sup> has also shown that the maximum pressures may be higher at certain parts of the arterial tree than at the root of the aorta. In order to explain his findings, O. Frank has compared the pulse in the peripheral arteries with the pressure records obtained by a manometer of relatively slow vibration period. Such a manometer will deform the original pulse picture by imparting its own oscillation time to the waves, and it is probable that the elastic arteries will have a similar effect on the pulse which comes to them. According to Frank, the wave motion in the arteries is more readily understood if it be compared with the stationary waves of a relatively short closed elastic system than if it be compared to the waves which travel along elastic tubes of great length.

The hypothesis which we have adopted to explain the marked negative flow toward the end of the systolic period of the nitroglycerin pulse is in accord with Frank's views. This hypothesis is as follows: When nitroglycerin is given a large primary wave enters the relaxed brachial artery and passes down its branches; but since only a small portion of this wave can escape through the unrelaxed arterioles and capillaries, the larger arterial trunks and their branches become distended, and through their elastic recoil the wave is returned toward the heart. According to this hypothesis, the immediate reflection of the primary wave from various points in the arterial tree becomes of less importance than the reflection by the distention and elastic recoil of the arterial walls. To make the conception more clear, one may compare the reflection of pulse waves with the reflection of an air wave, which enters a resonator of the general type of a flask with a relatively narrow neck. When a sudden wave of air is thrown into such a flask it is not immediately reflected as

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25. Dawson, P.: The Lateral Blood "Pressures" at Different Points of the Arterial Tree. *Am. Jour. Physiol.*, 1906, xv, 244.

it would be from a plane surface, but it causes a compression of the air within the flask, and this is reflected by the "elastic recoil" of the compressed air. Its period of oscillation is far longer than if the wave had entered a closed pipe which did not open out into a flask. We believe, therefore, that the reflection of the primary wave from the periphery, which is such a prominent feature of the nitroglycerin pulse, is due in the main to an elastic recoil of the larger arteries and their branches.

Even under normal circumstances a negative wave of blood-flow in the brachial artery frequently succeeds the primary pulse wave. We believe that this is to be explained on the same principle that we have used to explain the occurrence in the nitroglycerin pulse: viz., by a reflection of the main pulse wave due to the elastic recoil of the arteries. The peculiar features of the nitroglycerin pulse may all be explained by assuming a reduction in the tonus of the larger arteries and their branches.

#### CONCLUSIONS

1. An augmentation of the arm volume does not necessarily indicate an increase in the local blood-flow. This is seen in the nitroglycerin pulse.

2. The volume pulse is no reliable index of changes in the blood-flow through the arm. In different pathological conditions the two bear no relation to each other. In a single individual the volume pulse is relatively small when very fast flows are produced by heat and by exercise, and it is relatively large after nitroglycerin has been given.

3. A slow back-flow toward the heart during the diastolic period of the pulse is indicated in some of our normal tracings of the brachial pulse flow. This is not common, however, and it may be due in some cases at least to technical errors.

4. A peripheral reflection of the primary pulse wave due to the elastic recoil of the peripheral arteries may produce a negative wave of flow in the brachial artery during late systole. This is a very prominent feature of the nitroglycerin pulse.

#### III. AORTIC INSUFFICIENCY

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In some patients with physical signs of aortic insufficiency the arterial pulse shows but few changes from the normal. In its typical form, however, the arterial pulse of aortic insufficiency is characterized by a large and sudden rise (*pulsus magnus et celer*, water hammer pulse) and by a rapid fall (collapsing pulse). The arteries are often tortuous and of large caliber. The diastolic pressure is low and the difference between sys-

tolic and diastolic pressures is markedly increased: so that these changes in the pressures constitute an important sign of the disease. In typical cases the sphygmogram of the radial pulse is unusually large. According to Mackenzie and Broadbent the most characteristic feature of the radial pulse is the rapid fall which takes place before the occurrence of the dicrotic notch. Stewart<sup>26</sup> had pointed out that since the dicrotic notch marks the end of ventricular systole this fall previous to the notch cannot depend directly on a regurgitant wave into the ventricle. Mainly on the basis of animal experiments he has advanced the view that there is a reflex inhibition of the vasomotor center which increases the flow through the capillaries and so lowers the blood-pressure even during the latter part of the cardiac systole. According to Stewart, the amount of blood which regurgitates into the ventricle is inconsiderable and the fall of diastolic pressure is mainly dependent on the increased flow through the capillaries. MacCallum<sup>27</sup> has studied the effect of aortic regurgitation on the pulse form and the pressures when an artificial (rubber) aorta is connected to the living heart of a dog. His measurements indicate that while the amount of blood which regurgitates into the ventricle in artificially produced aortic insufficiency is small when expressed in cubic centimeters it may be large relative to the ventricular output. MacCallum's method of experiment enabled him to measure directly the outflow from his artificial aorta and he was thus able to demonstrate that the pulse form and pressure changes characteristic of aortic insufficiency did not in his experiments depend on an increased outflow. As a result of these experiments, he concluded that in aortic insufficiency the regurgitation of blood into the ventricle produced the low diastolic pressure, that the extensive and violent excursions of the ventricle produced the large pulse pressure, and that the low diastolic tension of the arterial wall allowed a large systolic "fling" which accounted for a large part of the high pulse wave and for the low position of the dicrotic notch. It seems to us that the objections which might be raised to MacCallum's experiments relate mainly to the "fling" which he describes. Did this fling in his tracings originate in his recording apparatus or in the rubber tube used to replace the aorta, and if it originated in the latter, may one assume a similar "fling" in the arterial system of man?

We have approached this subject from the study of the brachial flow pulse as it occurs in the aortic insufficiency of man. It is true as MacCallum has pointed out that in man the conditions may be modified by secondary changes in the arteries themselves, and that the characteristic

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26. Stewart, H. A.: Experimental and Clinical Investigation of the Pulse and Blood-Pressure Changes in Aortic Insufficiency. *THE ARCHIVES INT. MED.*, 1908, i, 102. (Literature reviewed.)

27. MacCallum, W. G.: The Changes in the Circulation in Aortic Insufficiency. *Bull. Johns Hopkins Hospital*, 1911, xxii, 197.

pulse in man may not be due entirely to the valve lesion. However, since the chief interest in the subject centers about the collapsing pulse as observed clinically, it is of prime importance that one should obtain a clear conception of the collapsing pulse encountered in man. The following questions therefore present themselves: Is there an appreciable back-flow in the brachial artery during diastole in typical instances of the collapsing pulse? Is the fall of pressure which has been recorded during the latter part of systole due to a rapid escape of blood through the capillaries or is it due to an arterial "fling"?

#### THE PRIMARY WAVE IN COLLAPSING PULSES

The following study of the brachial pulse flow in aortic insufficiency is based on tracings obtained from seven patients who showed all degrees of the vascular phenomena characteristic of this lesion. This list includes all patients showing marked vascular phenomena who presented themselves at the medical clinic during the past eight months, but omits four or five with diastolic murmurs unaccompanied by definite vascular phenomena, for the reason that little change from the normal was to be expected in such cases. In the following table the cases are arranged according to the size of the primary pulse waves in the brachial artery as measured above the average inflow.

TABLE 3.—PRIMARY WAVE IN COLLAPSING PULSES. CASES ARRANGED ACCORDING TO SIZE OF PRIMARY PULSE WAVES IN THE BRACHIAL ARTERY AS MEASURED ABOVE AVERAGE INFLOW

Name	Collapsing Quality	Capill. Pulse	Duroziez's Sign	Brachial Pressures	Area Heart Shadow	Height Primary Wave	Flow Per Beat	Flow per 100 c.c. Arm Per Minute
Hol. ...	+	++	+	150	...	2.5	2.0	10.0
Nei. ...	+	++	+	138-25	123	2.1	0.5	2.3
Wid. ...	+	+	+	142-45	134	2.0	0.5	3.3
Wood ..	+	+	+	120-48	118	1.4	0.5	2.3
Br. ....	+	+	+	132-50	...	1.4	1.1	9.3
Wel. ...	?-0	0	0	.....	121	0.8	1.0	3.1
Cl. ....	0	0	?	158	116	0.6	0.4	3.8

It will be seen that in five of the seven cases the vascular phenomena were clinically well marked and in two they were absent or slight. In all of the former the primary wave equalled or exceeded 1.4 c.c. of blood. This large primary wave is characteristic of the marked vascular phenomena of aortic insufficiency, for in no other condition have we encountered primary waves which exceeded 1.1 c.c., while the usual size of the primary wave is from 0.4 to 0.9 c.c. The causes of a large primary wave have already been enumerated in the discussion of the large wave of the nitroglycerin pulse. In brief they are (1) a large or sudden systolic output from the heart, and (2) a relaxed condition of the



peripheral arteries which allows a large pulse wave to enter them. Both factors probably play a part in the production of the unusually large primary wave which seems to be characteristic of collapsing pulses.

#### THE DIASTOLIC BACK-FLOW IN THE BRACHIAL ARTERY

We have already mentioned the fact that a slight continuous back-flow during the diastolic period of the pulse may be indicated by our tracings from normal individuals. This is rather uncommon, however, and it is always a slow movement. In the case of the collapsing pulse, the diastolic back-flow may be very rapid, and in some of our tracings three-fourths of the blood which entered the brachial during the primary wave left again during the remainder of the pulse cycle (Fig. 5). The actual amount of back-flow depends in part on the amount of blood which is allowed to pass through the arterioles and capillaries into the veins.

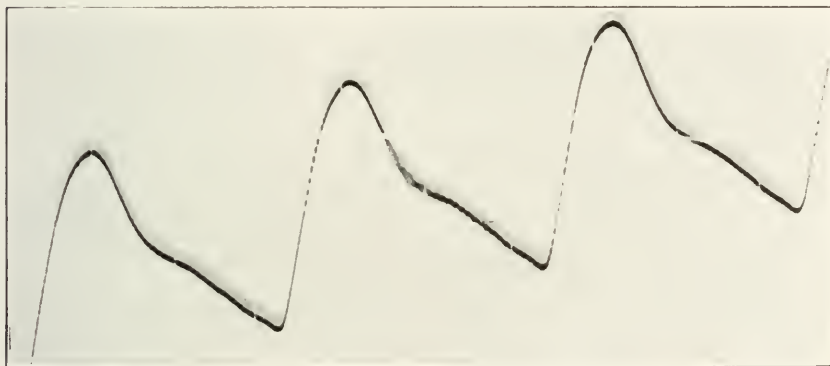


Fig. 5.—The pulse flow in a markedly collapsing pulse of aortic insufficiency. Average blood-flow 3.6 c.c. per minute. The primary wave put in 2 c.c. above the average rate of flow. The total amount of blood entering with one complete pulse cycle was 0.5 c.c.

Should the peripheral resistance in the arm be reduced then a large part of the blood which enters with the primary wave may pass on into the capillaries and veins, leaving but little to be returned toward the heart in diastole. Such a condition is not the rule, however, and it is certain that the collapsing character of the pulse has nothing to do with the amount of resistance offered by the local arterioles and capillaries of the arm. It was present whether the blood-flow in the arm was rapid or slow (Table 3).

It is possible to demonstrate in another way that the diastolic fall of pressure in aortic insufficiency does not depend on a rapid flow through the capillaries of the arm. Owing to the low diastolic pressure in this condition, a relatively low pressure must be used in the cuff that we use to constrict the veins just outside of the plethysmograph. In aortic

insufficiency pressures of 40 to 70 mm. of mercury may usually be used without markedly deforming the flow tracings. When higher pressures (70 to 120 mm.) are used, an interesting deformity appears on the tracings. As will be seen in Figure 6, the result is a reduction in the height of the primary wave and a coincident reduction in the back-flow during the latter part of the pulse tracing. The rate of blood-flow to the arm remains practically unchanged. The higher pressure in the cuff has cut off the arterial inflow and arterial outflow to about the same extent. Had the marked fall in diastolic pressure been due to a rapid flow through the arterioles and capillaries of the arm, one would have anticipated that a rise of pressure in the cuff above the diastolic pressure would have interrupted this onflow and have slowed the rate of flow to the arm.

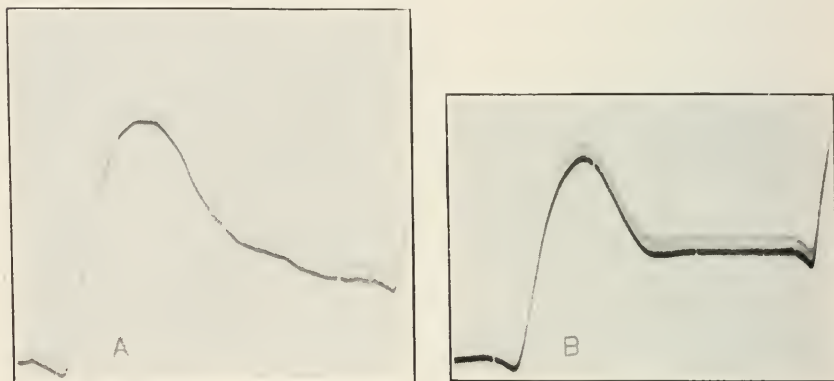


Fig. 6.—Examples of the deformity produced in the collapsing pulse by high pressures in the constricting cuff outside of the plethysmograph. In A a pressure of 90 mm. Hg was applied and in B a pressure of 118 mm. From the same patient as Figure 5. The average rate of flow in the arm was not diminished in spite of the deformity of the curve produced by the high pressure.

From our tracings, therefore, we conclude that in aortic insufficiency there is a marked back-flow in the brachial artery during the diastolic portion of the pulse cycle. This back-flow in the brachial artery is not dependent on gravity, for it has been obtained even when the arm is lowered below the general level of the body. Furthermore, this back-flow is not limited to the brachial artery, for in these patients a diastolic murmur can be produced in the femoral artery by a proper degree of compression. This phenomenon, Duroziez's sign, is difficult to explain in any other way than by assuming a back-flow in the femoral artery during diastole.

One might attempt to explain the very marked diastolic back-flows frequently seen in the brachial arteries of patients with the collapsing

pulse by assuming an extremely free flow of blood through the internal organs. Yet a similar degree of diastolic back-flow has not been encountered in any other condition, whereas it is the rule in the collapsing pulses of aortic insufficiency. It seems to us quite certain, therefore, that the marked diastolic back-flow is due to the regurgitant leak through the aortic valves and that when marked it is pathognomonic of this valve lesion.

#### PERIPHERAL REFLECTION IN AORTIC INSUFFICIENCY

There remains for consideration the well-marked back-flow in the brachial artery which extends from the end of the primary wave to the beginning of the dicrotic wave. If we assume that the latter corresponds approximately to the closure of the semilunar valves, then, as Stewart has pointed out, it is not possible to explain this back-flow as being due directly to the regurgitation of blood into the ventricle. It seems to us evident that this systolic back-flow in aortic insufficiency is in every way comparable to the similar back-flow of the nitroglycerin pulse and that like the latter, it depends on an elastic reflection of the primary pulse wave from the periphery. Indeed, the conditions in aortic insufficiency are peculiarly favorable for such a reflection, for a large quantity of blood is expelled by the ventricle into a vascular system already relaxed by the regurgitant leak. The large pulse wave travels through the relaxed arteries to the obstruction encountered in the finer arterioles and capillaries, and it may even enter the latter producing the capillary pulse. Our flow tracings, however, show that but a small proportion of the wave is allowed to pass the capillaries. The major portion distends the large arteries. A part is immediately thrown back by their elastic recoil, while another part is often returned toward the heart during diastole.

#### CONCLUSIONS

1. The brachial pulse flow in typical cases of the collapsing pulse of aortic insufficiency is characterized by (1) the large size of the primary wave, (2) by the common occurrence of a rapid back-flow during diastole and (3) by a marked reflection of the primary pulse wave.

2. The large primary wave depends in part on the unusual amount of blood expelled by the left ventricle during systole and in part on the relaxed condition of the arterial tree.

3. The back-flow during diastole is due mainly to the regurgitation into the left ventricle.

4. The back-flow during the latter portion of systole is due to an unusually marked reflection of the large primary pulse wave, produced by an elastic recoil of the peripheral arteries.

## THE EFFECT OF TEMPORARY OCCLUSION OF RENAL CIRCULATION ON RENAL FUNCTION \*

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BALTIMORE

Of great importance in relation to the surgery of the kidney is the question concerning the length of time that the renal vessels may be clamped without permanent injury to renal function or renal tissue. The question is dealt with here experimentally. The renal vessels in rabbits and dogs have been clamped for varying periods of time, and subsequently renal function has been tested by the excretion of phenolsulphonephthalein, lactose, potassium iodid, salt and water. The presence or absence of albumin and casts in the urine has been observed. The results of such functional studies have been controlled by histological examination of the kidneys.

The effect of obstruction to the renal circulation on the urinary picture and the histology of the kidney has already been investigated. Robinson<sup>1</sup> showed that tying of the renal vein resulted in the appearance of albumin, blood, or both, in the urine, and in enlargement of the kidney. This has been confirmed by other observers. Paneth,<sup>2</sup> Munk<sup>3</sup> and de Souza<sup>4</sup> have shown that following ligation of the renal vein, the urine is small in amount. Ludwig<sup>5</sup> found that clamping the renal vessels for a period of minutes frequently interfered with the secretion of urine for hours. Heidenhain<sup>6</sup> found that on freeing the vessels after a temporary clamping the secretion of urine did not begin at once. Litten<sup>7</sup> observed after clamping both renal vessels for periods up to two hours, that the kidney became enlarged and congested. If the renal artery alone was tied for one-half or one hour, albumin and casts appeared in the urine. Pathologically, the tubules were affected as shown by their

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\* Submitted for publication March 20, 1913.

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\* For review of literature concerning partial or complete obstruction to renal circulation see our previous paper. Rowntree, Fitz and Geraghty: Studies of Renal Function in Experimental Chronic Passive Congestion, THE ARCHIVES INT. MED., 1913, xi, 121.

1. Robinson: Med. Chir. Tr., 1843. xxvi, 51.

2. Paneth: Pflüger's Arch., 1886. xxxix. 515.

3. Munk: Berl. klin. Wchnschr., 1864, i. 333.

4. De Souza: Jour. Physiol., 1900. xxvi, 139.

5. Ludwig Physiologie, 1856. ii, 416.

6. Heidenhain: Hermann's Handb. d. Physiol., 1883, v. Teil 1, 321.

7. Litten: Ztschr. f. klin. Med., 1880. p. 131.



inability to stain with indigo carmin. But such changes were temporary. If the artery was clamped for two hours, permanent injury occurred, evidenced by tubular necrosis with cast production, cellular infiltration, resulting in circumscribed scar formation with calcification.

Chirié and Mayer<sup>8</sup> after temporary clamping of both renal veins in dogs, noted, in four out of seven cases, death with convulsions. Carrel<sup>9</sup> was unable to confirm this observation.

Eisendrath and Strauss<sup>10</sup> have studied the pathological changes in the kidneys of rabbits after compressing the renal vessels for from fifteen to ninety minutes. According to their results, temporary compression — half an hour or less — caused slight damage. If longer, marked permanent lesions occurred in the parenchyma, as evidenced by interstitial cellular infiltration, coagulation necrosis of tubular epithelium, and later by the deposition of calcium in and about the destroyed epithelium.

Thus it has been shown that clamping of the renal vessels for periods up to an hour may produce temporary anuria, albuminuria and cylindruria. If interference with the renal circulation is maintained sufficiently long, permanent interstitial increase in tissue occurs in the kidney. Except for the amount of urine excreted and the presence in it of albumin or casts, no systematic study of renal function under such conditions has been made.

#### AUTHORS' EXPERIMENTS

In our experiments rabbits and dogs were etherized and the abdomen opened aseptically in the median line. The left kidney was exposed, and by careful blunt dissection the vessels were freed from the surrounding tissues. The artery and vein were raised on an artery forceps with as little stretching as possible and a small rubber protected bull-dog clamp placed around them. In each experiment it was noted that on the distal side of the forceps the artery did not pulsate, while the vein at once became distended; on the proximal side the vein was collapsed and the artery pulsated normally. No attempt was made to prevent the capsular circulation. After the left renal vessels were clamped, the right renal vessels were ligated. The right kidney was removed and weighed. In a few animals, for control, the right kidney was left untouched.

While the clamp was in place, the abdomen was closed and covered with a warm saline pad and towel. To try to obviate the factor of shock, the body heat was maintained. The animals were anesthetized as lightly as possible. After the desired interval of time had elapsed, the clamp was removed, the macroscopic appearance of the kidney noted, and

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8. Chirié and Mayer: *Compt. rend. Soc. de biol.*, 1907, i, 598.

9. Carrel: *Compt. rend. d. Soc. de biol.*, 1909, i, 527.

10. Eisendrath and Strauss: *Jour. Am. Med. Assn.*, 1910, iv, 2286; see also Guthrie: *THE ARCHIVES INT. MED.*, 1910, v, 232.

the peritoneum and abdomen sewn up. The bladder was then emptied and the animals placed in metabolism cages. The clamp was applied in series of animals for ten, twenty, thirty, forty and sixty minutes. Repeated studies of renal function were made. Finally, the animals were killed, submitted to autopsy and the kidneys examined histologically after formaldehyd fixation, celloidin imbedding and hematoxylin and eosin staining.

#### TECHNIC OF TESTS OF RENAL FUNCTION

A short description of the various tests used for renal function, with their technic, is given.

The phthalein test was made according to the original technic of Rowntree and Geraghty.<sup>11</sup> One cubic centimeter of a phenolsulphonephthalein solution containing accurately 6 milligrams was injected aseptically into the leg muscles of the rabbits or lumbar muscles of the dogs, which were then placed in metabolism cages. The bladders were expressed or catheterized at the end of an hour and ten minutes, and the total urine for this time collected. The urine was made distinctly alkaline, diluted to 1 liter, and the amount of drug determined by the use of Rowntree and Geraghty's<sup>12</sup> modification of the Autenrieth-Königsberger colorimeter. In control animals it has been determined that the normal output in this time is 60 per cent. or more.

The lactose, potassium iodid, salt and water tests which have received a thorough study at the hands of Schlayer<sup>13</sup> and his co-workers and which we have used in relation to the renal function in experimental and clinical nephritides, were applied to this study.

From our previous work we feel that the mechanism of the excretion of lactose differs essentially from that of phthalein, salt and iodid. Throughout this investigation we have used it as an index of the condition of the vascular function<sup>14</sup> of the kidney, admitting that we need much more information concerning the manner and significance of its excretion.

The technic for the lactose test in rabbits has been identical with Schlayer's. One gram of lactose dissolved in 10 c.c. of distilled water was injected into the ear vein. The animals were placed in metabolism cages, the bladder expressed at the end of four hours and every hour thereafter up to eight hours. In dogs, according to our previous technic, 2 gm. were dissolved in 20 c.c. of water, and injected into the lumbar muscles.

Since the time necessary for total elimination has been considered by Schlayer of greater importance than the absolute amount recovered, and since our previous observations with lactose excretion agree with this, we have observed this exclusively. The presence of lactose in the urine was determined by Nylander's test, using similar amounts of urine, reagent and length of boiling time.

In rabbits under these conditions, the time necessary for the complete elimination of lactose is normally six hours or less and in dogs from four to six hours.

11. Rowntree and Geraghty: *Jour. Phar. and Exper. Therap.*, 1910, i, 579.

12. Rowntree and Geraghty: *THE ARCHIVES INT. MED.*, 1912, ix, 284.

13. Schlayer: *Deutsch. Arch. f. klin. Med.*, 1911, cii, 311; Schlayer and Takayasu: *Deutsch. Arch. f. klin. Med.*, 1891, xcviii, 17; 1910, ci, 333.

14. For a review of the literature on this test and the others described below, see our previous paper. We have shown in a previous publication, however, that lactose is not excreted solely by the glomeruli.

According to the studies of Schlayer, potassium iodid is excreted by the tubules of the kidney, and on it he has placed most dependence in determining tubular functional capacity. In these studies 1 c.c. of a 2.5 per cent. solution has been administered intravenously to rabbits. For this the normal elimination time is twenty-four hours. In dogs, 0.5 gm. has been administered by mouth, which normally is excreted within sixty hours. The presence of the drug in the urine has been determined by Sandow's<sup>15</sup> test.

The excretion of salt following its administration in amounts greatly in excess of that ordinarily taken with the food is accomplished by the tubules, according to Schlayer. Normally, a large amount of salt is excreted by one of two methods. If it is given without extra water, it is almost entirely excreted within twenty-four hours without diuresis by increased salt concentration in the urine; if given with an excess of water, it is excreted partially through increased concentration in the urine and partially through diuresis.

Where vascular injury to the kidney exists, the simple administration of salt may be followed by a marked diuresis, all of the salt being excreted in twenty-four hours without its percentage content in the urine being at all increased. This is usually associated with a somewhat low and fixed specific gravity and the syndrome is spoken of as "vascular hypostenuria." Here the inability to concentrate is not due to any incapacity of the tubules to excrete salt, but to hypersensitive vessels which respond to the salt administration with a diuresis. In more severe vascular injury the vessels do not act in the same way, oliguria characterizing the urinary picture. In severe tubular destruction, a urine of fixed low specific gravity is obtained, the quantity of which is not materially affected by the administration of extra amounts of salt, and the salt content of which is not augmented by administration of extra amounts of salt because of the inability of the tubules to excrete it. Such a condition is known as "tubular hypostenuria."

In these studies gm. 1.50 were given by stomach-tube to the rabbits, and gm. 3 to dogs. The urine for the following twenty-four hours was collected, and the salt concentration and absolute excretion estimated by the Lütke-Martius<sup>16</sup> method.

Finally, in order to make conditions as constant as possible, the rabbits were given 100 c.c. of water daily by stomach-tube. In addition,

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15. Sandow's method consists in adding 1 c.c. of 2 per cent. sodium nitrite solution and 1 c.c. of 10 per cent.  $H_2SO_4$  to from 10 to 30 c.c. of urine, followed by the addition of a small amount of chloroform. This is shaken together and allowed to separate into layers, the presence of the iodid being indicated by a purplish red or violet color in the chloroform.

16. Sähli: *Lehrbuch der klinischen untersuchungen, Methoden*, Franz Deuticke, Leipzig and Wien, 1899, p. 421.

they were allowed to eat green vegetables *ad libitum*. This produced a constant polyuria. Hence the amounts of urine were not followed from day to day. The dogs were allowed 300 c.c. of water daily, and were fed on meat when able to retain liquid or solid food. The lactose, phthalein and iodid tests were given synchronously since their excretion, and quantitative determination do not interfere with each other. In dogs, lactose and phthalein were given at the same time, but into the muscles of opposite sides.

Single catheter or expressed specimens of urine were taken from day to day. Albumin was tested by the nitric acid method. The sediment of centrifuged urine was examined microscopically for blood or casts.

As previously, in these studies we have considered the excretion of lactose as an index of vascular functional capacity; that of phthalein as an index of total renal function (though predominantly tubular), and the excretion of salt and iodid as an index of tubular functional capacity.

The results of these studies are shown in the accompanying protocols.<sup>17</sup>

#### PROTOCOLS OF EXPERIMENTS

I. Animals with one kidney removed, renal circulation clamped for 10 minutes.

RABBIT 1.—Body weight 1,650 gm. Weight of removed kidney 4.9 gm. Weight of remaining kidney at death 6.5 gm.

Sulphonephthalein excretion second day after operation 37 per cent.

Sulphonephthalein excretion third day after operation 62 per cent.

Sulphonephthalein excretion fifth day after operation 53 per cent.

Sulphonephthalein excretion seventh day after operation 58 per cent.

Lactose excretion first day after operation 6-7 hours.

Lactose excretion fourth day after operation 6-7 hours.

Iodid excretion first day after operation 36 hours +.

Iodid excretion fifth day after operation 36 hours +.

Salt excretion third day after operation 1.00 per cent. (1.20 gm.)

Salt excretion sixth day after operation 1.30 per cent. (1.00 gm.)

Albumin present for four days. Rare hyaline casts found until the seventh day. Animal killed on the eighth day.

RABBIT 2.—Weight 1,250 gm. Weight of removed kidney 4.6 gm. Weight of remaining kidney at death 4.5 gm.

Sulphonephthalein excretion second day after operation 65 per cent.

Sulphonephthalein excretion fourth day after operation 65 per cent.

Sulphonephthalein excretion fifth day after operation 62 per cent.

Sulphonephthalein excretion seventh day after operation 65 per cent.

Lactose excretion first day after operation 6 hours.

Lactose excretion fourth day after operation 7½ hours.

Lactose excretion seventh day after operation 5 hours.

Iodid excretion first day after operation 30 hours.

Iodid excretion fourth day after operation 24 hours.

Iodid excretion seventh day after operation 36 hours.

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17. In addition seven rabbits were used, one with circulation clamped for ten minutes, three for thirty minutes, and two for forty minutes. All died within sixteen hours of operation. Realizing the individual variation in susceptibility and vitality of rabbits, it seemed fair to exclude these animals from their appropriate tables, especially as all had been deeply anesthetized, all but one had made poor ether recoveries, and one rabbit at operation had suffered from a severe hemorrhage due to trauma of the liver.



Salt excretion third day after operation 1.25 per cent. (1.00 gm.)  
 Salt excretion sixth day after operation 1.3 per cent. (1.3 gm.)  
 Albumin present for six days. Sediment negative throughout the experiment.  
 Animal found dead on the eighth day. No cause found.

RABBIT 3.—Weight 2,250 gm. Weight of removed kidney 6 gm. (approx.).  
 Weight of remaining kidney at death 8 gm.

Sulphonephthalein excretion first day after operation 9 per cent.  
 Sulphonephthalein excretion second day after operation 24 per cent.  
 Sulphonephthalein excretion third day after operation 35 per cent.  
 Sulphonephthalein excretion sixth day after operation 62 per cent.  
 Sulphonephthalein excretion eighth day after operation, 80 per cent.

Lactose excretion first day after operation 8 hours +.

Lactose excretion sixth day after operation 6 hours.

Lactose excretion thirteenth day after operation 5 hours.

Iodid excretion first day after operation 30 hours.

Iodid excretion sixth day after operation 34 hours.

Salt excretion third day after operation .72 per cent. (1.35 gm.)

Salt excretion eighth day after operation .56 per cent. (1.40 gm.)

Albumin present for three days. Sediment showed rare hyaline casts on the thirteenth day. Hematuria for three days. The animal was killed on the fourteenth day.

II. Animals with one kidney removed. Renal circulation clamped for twenty minutes.

RABBIT 1.—Weight 1,800 gm. Weight of removed kidney 6.6 gm. Weight of remaining kidney at death 5.5 gm.

Sulphonephthalein excretion first day after operation 32 per cent.

Sulphonephthalein excretion third day after operation 43 per cent.

Sulphonephthalein excretion fifth day after operation 42 per cent.

Sulphonephthalein excretion thirteenth day after operation 48 per cent.

Sulphonephthalein excretion twentieth day after operation 37 per cent.

Lactose excretion first day after operation 7½ hours.

Lactose excretion fifth day after operation 6 hours.

Lactose excretion fourteenth day after operation 5 hours.

Lactose excretion twentieth day after operation 7 hours +.

Iodid excretion first day after operation 24 hours.

Iodid excretion fourteenth day after operation 24 hours.

Iodid excretion twentieth day after operation 24 hours.

Salt excretion third day after operation 1.00 per cent. (1.1 gm.)

Salt excretion seventh day after operation .8 per cent. (2.00 gm.)

Salt excretion sixteenth day after operation .7 per cent. (1.6 gm.)

Albumin present for seven days. Few hyaline casts found until the seventh day. Hematuria for five days. Animal found dead on the twenty-seventh day. Histologically the kidney not remarkable.

RABBIT 2.—Weight 1,720 gm. Weight of removed kidney 6.4 gm. Weight of remaining kidney at death 6.5 gm.

Sulphonephthalein excretion first day after operation 27 per cent.

Sulphonephthalein excretion fifth day after operation 32 per cent.

Sulphonephthalein excretion sixth day after operation 60 per cent.

Sulphonephthalein excretion fourteenth day after operation 58 per cent.

Sulphonephthalein excretion twentieth day after operation 58 per cent.

Sulphonephthalein excretion twenty-eighth day after operation 64 per cent.

Lactose excretion first day after death 7½ hours.

Lactose excretion fifth day after operation 7 hours.

Lactose excretion fourteenth day after operation 5 hours.

Lactose excretion twenty-eighth day after operation 5 hours.

Iodid excretion first day after operation 24 hours.

Iodid excretion fifth day after operation 30 hours.

Iodid excretion fourteenth day after operation 24 hours.

Iodid excretion twenty-eighth day after operation 24 hours.

Salt excretion third day after operation .6 per cent. (1.1 gm.).

Salt excretion seventh day after operation 1.2 per cent. (1.8 gm.).

Salt excretion sixteenth day after operation .53 per cent. (1.3 gm.).

Salt excretion twenty-eighth day after operation .46 per cent. (2.00 gm.).

Albumin present for three days. Hematuria for four days. No casts found after the fifth day. Animal killed the twenty-eighth day.

RABBIT 3.—Weight 2,020 gm. Weight of removed kidney 6.5 gm. Weight of remaining kidney at death 7.00 gm.

Sulphonaphthalein excretion first day after operation 55 per cent.

Sulphonaphthalein excretion third day after operation 35 per cent.

Sulphonaphthalein excretion fifth day after operation 65 per cent.

Sulphonaphthalein excretion fifteenth day after operation 64 per cent.

Sulphonaphthalein excretion twenty-eighth day after operation 70 per cent.

Lactose excretion first day after operation 7½ hours.

Lactose excretion fifth day after operation 4 hours.

Lactose excretion fifteenth day after operation 4 hours.

Lactose excretion twenty-third day after operation 6 hours.

Iodid excretion first day after operation 24 hours.

Iodid excretion fifth day after operation 24 hours.

Iodid excretion twenty-third day after operation 24 hours.

Salt excretion third day after operation 1.00 per cent. (1.1 gm.).

Salt excretion twelfth day after operation .8 per cent. (2.4 gm.).

Albumin present for three days. Hematuria for one day. No casts found after the sixth day. Animal killed the twenty-eighth day.

DOG 1.—Weight 5 kilos. Weight of the removed kidney 20 gm.

Sulphonaphthalein excretion first day after operation 40 per cent.

Sulphonaphthalein excretion second day after operation 60 per cent.

Sulphonaphthalein excretion fifth day after operation 50 per cent.

Sulphonaphthalein excretion ninth day after operation 68 per cent.

Sulphonaphthalein excretion twelfth day after operation 60 per cent.

Lactose excretion first day after operation 8 hours +.

Lactose excretion fifth day after operation 8 hours.

Lactose excretion ninth day after operation 5 hours.

Iodid excretion second day after operation 48 hours.

Iodid excretion twelfth day after operation 48 hours.

Salt excretion second day after operation .85 per cent. (3.00 gm.).

Albumin present for one day. No casts found after the second day. The animal was allowed to live, since he had made an apparently normal recovery.

III.—Animals with one kidney removed; renal circulation clamped for thirty minutes.

RABBIT 1.—Weight 1,700 gm. Weight of removed kidney 5 gm. (Approx.) Weight of remaining kidney after death 6.5 gm.

Sulphonaphthalein excretion first day after operation 46 per cent.

Sulphonaphthalein excretion second day after operation 40 per cent.

Sulphonaphthalein excretion fourth day after operation 60 per cent.

Sulphonaphthalein excretion thirteenth day after operation 67 per cent.

Sulphonaphthalein excretion twenty-eighth day after operation 72 per cent.

Sulphonaphthalein excretion thirty-first day after operation 60 per cent.

Lactose excretion first day after operation 6½ hours.

Lactose excretion fourth day after operation 5 hours.

Lactose excretion thirteenth day after operation 5 hours.

Lactose excretion twenty-eighth day after operation 5 hours.

Iodid excretion first day after operation 36 hours.

Iodid excretion fourth day after operation 30 hours.

Iodid excretion thirteenth day after operation 24 hours.

Salt excretion third day after operation 1.3 per cent. (1.7 gm.).

Salt excretion sixth day after operation .8 per cent. (1.4 gm.).

Salt excretion thirty-first day after operation .9 per cent. (1.4 gm.).

Albuminuria for four days. No casts found after the thirteenth day. Animal killed on the thirty-first day.

RABBIT 2.—Weight 2,400 gm. Weight of removed kidney 7.6 gm. Weight of remaining kidney after death 11.5 gm.



Sulphonephthalein excretion first day after operation 36 per cent.  
 Sulphonephthalein excretion second day after operation 40 per cent.  
 Sulphonephthalein excretion fourth day after operation 70 per cent.  
 Sulphonephthalein excretion fifteenth day after operation 62 per cent.  
 Lactose excretion first day after operation 7 hours +.  
 Lactose excretion fourth day after operation 5 hours.  
 Lactose excretion fifteenth day after operation 5 hours.  
 Iodid excretion fifteenth day after operation 24 hours.  
 Salt excretion first day after operation .7 per cent. (1.6 gm.).  
 Salt excretion fifteenth day after operation .6 per cent. 2.4 gm.).

Albumin present for one day. Hematuria for two days. No casts found after the fourth day. Animal killed the fifteenth day.

RABBIT 3.—Weight 2,600 gm. Weight of removed kidney 9.6 gm. Weight of remaining kidney after death 8 gm.

Sulphonephthalein excretion second day after operation 55 per cent.  
 Sulphonephthalein excretion fourth day after operation 70 per cent.  
 Sulphonephthalein excretion fifteenth day after operation 80 per cent.  
 Sulphonephthalein excretion twentieth day after operation 74 per cent.  
 Lactose excretion first day after operation 7 hours.  
 Lactose excretion fourth day after operation 5 hours.  
 Lactose excretion fourteenth day after operation 7 hours.  
 Lactose excretion twentieth day after operation 5 hours.  
 Iodid excretion first day after operation 24 hours.  
 Iodid excretion fourth day after operation 24 hours.  
 Iodid excretion fifteenth day after operation 24 hours.  
 Salt excretion third day after operation 1.1 per cent. (2.5 gm.).  
 Salt excretion fourteenth day after operation .52 per cent. (2.1 gm.).  
 Salt excretion twentieth day after operation .65 per cent. (1.9 gm.).

Albumin present for one day. Hematuria for one day. No casts seen after the third day. Animal killed the thirtieth day.

DOG 1.—Weight 7 kilos. Weight of removed kidney 24 gm.

Sulphonephthalein excretion second day after operation 36 per cent.  
 Sulphonephthalein excretion third day after operation 52 per cent.  
 Sulphonephthalein excretion fifth day after operation 55 per cent.  
 Lactose excretion third day after operation 8 hours.  
 Iodid excretion second day after operation 48 hours.  
 Salt excretion third day after operation 1 per cent. (1.6 gm.).

Albumin for two days. Casts found on the fifth day. Animal made an uneventful recovery and allowed to live.

IV.—Animal with one kidney removed; renal circulation clamped for forty minutes.

RABBIT 1.—Weight 2,070 gm. Weight of removed kidney 5.7 gm. Weight of remaining kidney after death 7.2 gm.

Sulphonephthalein excretion first day after operation 24 per cent.  
 Sulphonephthalein excretion second day after operation 46 per cent.  
 Sulphonephthalein excretion third day after operation 50 per cent.  
 Sulphonephthalein excretion fourth day after operation 62 per cent.  
 Sulphonephthalein excretion eighth day after operation 70 per cent.  
 Lactose excretion second day after operation 6 hours.  
 Lactose excretion eighth day after operation 6 hours.  
 Lactose excretion twelfth day after operation 5 hours.  
 Iodid excretion first day after operation 48 hours.  
 Salt excretion fourth day after operation 1 per cent. (1.5 gm.).  
 Salt excretion tenth day after operation .7 per cent. (1.2 gm.).

Albuminuria for eight days. Hematuria for one day. No casts found after the fourth day. Animal killed on the twelfth day on account of general infection.

RABBIT 2.—Weight 1,600 gm. Weight of removed kidney 4.6 gm. Weight of remaining kidney after death 6.5 gm.

Sulphonephthalein excretion first day after operation 46 per cent.  
 Sulphonephthalein excretion second day after operation 47 per cent.

Sulphonaphthalein excretion third day after operation 56 per cent.

Lactose excretion first day after operation 8 hours +.

Lactose excretion third day after operation 7 hours +.

Iodid excretion first day after operation 30 hours.

Iodid excretion third day after operation 24 hours.

Salt excretion third day after operation 1 per cent. (1.2 gm.)

Albumin and casts present until death. Animal killed on the fourth day on account of general infection.

RABBIT 3.—Weight 2,200 gm. Weight of removed kidney 6.00 gm. Weight of remaining kidney after death 9.00 gm.

Sulphonaphthalein excretion first day after operation 45 per cent.

Sulphonaphthalein excretion second day after operation 54 per cent.

Sulphonaphthalein excretion fourth day after operation 60 per cent.

Sulphonaphthalein excretion fifth day after operation 70 per cent.

Lactose excretion first day after operation 8 hours.

Lactose excretion third day after operation 5 hours.

Lactose excretion fifth day after operation 4 hours.

Iodid excretion first day after operation 30 hours.

Iodid excretion third day after operation 24 hours.

Salt excretion second day after operation .9 per cent. (1.00 gm.)

Salt excretion fifth day after operation .9 per cent. (2.5 gm.)

Albumin present for three days. Hematuria for one day. No casts seen after the fourth day. Animal killed on the sixth day.

RABBIT 4.—Weight 1,770 gm. Weight of removed kidney 5.00 gm. Weight of remaining kidney after death 7.00 gm.

Sulphonaphthalein excretion first day after operation 15 per cent.

Sulphonaphthalein excretion second day after operation 14 per cent.

Sulphonaphthalein excretion third day after operation 8 per cent.

Lactose excretion first day after operation 8 hours +.

Lactose excretion third day after operation 6 hours.

Iodid excretion first day after operation 30 per cent.

Salt excretion second day after operation 1.00 per cent. (.7 gm.)

No albumin found after the second day. Hematuria for one day. Casts seen on the third day. Animal found dead on the fourth day with acute peritonitis.

RABBIT 5.—Weight 2,320 gm. Weight of removed kidney 7 gm. Weight of remaining kidney after death 10 gm.

Sulphonaphthalein excretion first day after operation 47 per cent.

Sulphonaphthalein excretion fourth day after operation 62 per cent.

Sulphonaphthalein excretion twelfth day after operation 67 per cent.

Sulphonaphthalein excretion fourteenth day after operation 70 per cent.

Lactose excretion second day after operation 6 hours.

Lactose excretion fourth day after operation 6 hours.

Lactose excretion thirteenth day after operation 5 hours.

Iodid excretion second day after operation 24 hours.

Salt excretion second day after operation .7 per cent. (1.3 gm.)

Salt excretion thirteenth day after operation .9 per cent. (2.5 gm.)

Albuminuria for two days. Casts present for four days. Animal killed on the fifteenth day.

DOG 1.—Weight 6 kilos. Weight of removed kidney 33 gm. Weight of remaining kidney after death 50 gm.

Sulphonaphthalein excretion first day after operation traces.

Sulphonaphthalein excretion second day after operation traces.

Sulphonaphthalein excretion third day after operation traces.

Lactose excretion first day after operation 8 hours +.

Lactose excretion second day after operation 8 hours.

Salt and iodid not given. Albumin, blood and casts present until death.

Animal killed on the fourth day on account of extreme toxemia.

DOG 2.—Weight 10 kilos. Weight of removed kidney 30 gm.

Sulphonaphthalein excretion first day after operation 20 per cent.

Sulphonaphthalein excretion third day after operation 30 per cent.

Sulphonephthalein excretion fourth day after operation 64 per cent.  
 Sulphonephthalein excretion seventh day after operation 68 per cent.  
 Lactose excretion first day after operation 8 hours +.  
 Lactose excretion fourth day after operation 8 hours +.  
 Lactose excretion seventh day after operation 6 hours.  
 Iodid excretion second day after operation 48 hours.  
 Salt excretion second day after operation .4 per cent. (.80 gm.)

Albumin present for four days. No casts seen after the fourth day. Animal made an uneventful recovery and allowed to live.

V.—Animals with one kidney removed; renal circulation clamped for one hour.

RABBIT 1.—Weight 1,750 gm. Weight of removed kidney 7 gm. Weight of remaining kidney after death 7.5 gm.

Sulphonephthalein excretion first day after operation 32 per cent.  
 Sulphonephthalein excretion third day after operation 35 per cent.  
 Lactose excretion second day after operation 8 hours +.  
 Iodid excretion second day after operation 2 hours +.  
 Salt excretion second day after operation .6 per cent. (.5 gm.)

Albumin and casts found on the third day. Hematuria for one day. Animal found dead on the fourth day. Autopsy showed acute peritonitis.

RABBIT 2.—Weight 2,000 gm. Weight of removed kidney 5.2 gm. Weight of remaining kidney after death 6.5 gm.

Sulphonephthalein excretion first day after operation 7 per cent.  
 Lactose excretion second day after operation 8 hours +.  
 Salt excretion second day after operation .7 per cent. (.8 gm.)

Albumin, casts and blood in the urine on the third day. Animal found dead.

RABBIT 3.—Weight 1,800 gm. Weight of removed kidney 6.00 gm. Weight of remaining kidney after death 9.00 gm. Animal died anuric in seventy-two hours.

RABBIT 4.—Weight 1,700 gm. Weight of removed kidney 7 gm. Weight of remaining kidney after death 9.5 gm.

Sulphonephthalein excretion first day after operation 9 per cent.  
 Sulphonephthalein excretion second day after operation 12 per cent.  
 Sulphonephthalein excretion third day after operation 20 per cent.  
 Sulphonephthalein excretion sixth day after operation 8 per cent.  
 Lactose excretion first day after operation 8 hours +.  
 Lactose excretion third day after operation 8 hours +.  
 Iodid excretion first day after operation 48 hours.  
 Salt excretion third day after operation .8 per cent. (2.1 gm.)

Albumin and casts present until death. Animal found dead a few days after last note. Phthalein excreted in traces, lactose markedly delayed.

RABBIT 5.—Weight 1,400 gm. Weight of removed kidney 4 gm. Weight of remaining kidney after death 9.5 gm.

Sulphonephthalein excretion first day after operation traces.  
 Sulphonephthalein excretion second day after operation traces.  
 Sulphonephthalein excretion third day after operation traces.  
 Lactose excretion first day after operation none recovered.

Albumin, blood and casts present until death on the fourth day. During the last twenty-four hours passed 10 c.c. of urine.

VI.—Animals in which the renal circulation has been clamped in one kidney for an hour, the other kidney being left untouched.

RABBIT 1.—Weight 1,650 gm. Weight of unclamped kidney at death 5.00 gm. Weight of clamped kidney at death 5.00 gm.

Sulphonephthalein excretion first day after operation 30 per cent.  
 Sulphonephthalein excretion second day after operation 52 per cent.  
 Sulphonephthalein excretion sixth day after operation 56 per cent.  
 Sulphonephthalein excretion eighth day after operation 75 per cent.  
 Sulphonephthalein excretion eighteenth day after operation 60 per cent.

Sulphonaphthalein excretion twenty-second day after operation 70 per cent.  
 Sulphonaphthalein excretion twenty-fifth day after operation 76 per cent.  
 Lactose excretion first day after operation 7 hours.  
 Lactose excretion fourth day after operation 6 hours.  
 Lactose excretion eighth day after operation 5 hours.  
 Lactose excretion fifteenth day after operation 6 hours.  
 Lactose excretion twenty-fifth day after operation 5 hours.  
 Iodid excretion first day after operation 24 hours.  
 Iodid excretion eighth day after operation 24 hours.  
 Iodid excretion twenty-second day after operation 24 hours.  
 Salt excretion sixth day after operation .5 per cent. (1.1 gm.).  
 Salt excretion twenty-fifth day after operation .76 per cent. (1.2 gm.)

Albumin present for two days. No casts found after the sixth day. Animal killed on the twenty-sixth day.

RABBIT 2.—Weight 2,200 gm. Weight of unclamped kidney at death 7.5 gm. (approx.). Weight of clamped kidney at death 7.5 gm.

Sulphonaphthalein excretion first day after operation 59 per cent.  
 Sulphonaphthalein excretion fifth day after operation 74 per cent.  
 Sulphonaphthalein excretion seventeenth day after operation 78 per cent.  
 Lactose excretion third day after operation 6 hours.  
 Lactose excretion tenth day after operation 7 hours +.  
 Lactose excretion eighteenth day after operation 6 hours.  
 Iodid excretion third day after operation 24 hours.  
 Iodid excretion eighteenth day after operation 24 hours.  
 Salt excretion eighteenth day after operation .3 per cent. (1.20 gm.)

Albumin present for one day. No casts seen after the fifth day. Animal killed on the nineteenth day.

RABBIT 3.—Weight 1,700 gm.

Sulphonaphthalein excretion first day after operation 50 per cent.  
 Sulphonaphthalein excretion third day after operation 58 per cent.  
 Sulphonaphthalein excretion seventh day after operation 70 per cent.  
 Lactose excretion third day after operation 6 hours.  
 Lactose excretion seventh day after operation 5 hours.  
 Iodid excretion first day after operation 24 hours.  
 Iodid excretion seventh day after operation 24 hours.  
 Salt excretion fifth day after operation .7 per cent. (1.4 gm.)

Albumin present for one day. Casts found on the seventh day. Animal found dead on the twelfth day. No cause found.

VII.—Animals in which one kidney has been removed, the circulation of the other being left untouched.

RABBIT 1.—Weight 1,900 gm. Weight of removed kidney 7 gm. Weight of remaining kidney after death 7.5 gm.

Sulphonaphthalein excretion first day after operation 50 per cent.  
 Sulphonaphthalein excretion second day after operation 65 per cent.  
 Sulphonaphthalein excretion fifth day after operation 60 per cent.  
 Sulphonaphthalein excretion ninth day after operation 70 per cent.  
 Lactose excretion first day after operation 5 hours.  
 Lactose excretion ninth day after operation 5 hours.  
 Lactose excretion sixteenth day after operation 6 hours.  
 Iodid excretion first day after operation 24 hours.  
 Iodid excretion sixteenth day after operation 24 hours.  
 Salt excretion third day after operation .5 per cent. (2.00 gm.)

Traces of albumin found for one day. Blood and casts found for one day. Animal killed on the twentieth day.

RABBIT 2.—Weight 2,200 gm. Weight of removed kidney 6 gm. Weight of remaining kidney after death 8.5 gm.

Sulphonaphthalein excretion first day after operation 69 per cent.  
 Sulphonaphthalein excretion second day after operation 70 per cent.  
 Sulphonaphthalein excretion twelfth day after operation 75 per cent.  
 Sulphonaphthalein excretion thirteenth day after operation 80 per cent.



Lactose excretion first day after operation 5 hours.  
 Lactose excretion thirteenth day after operation 5 hours.  
 Iodid excretion first day after operation 24 hours.  
 Iodid excretion thirteenth day after operation 24 hours.  
 Salt excretion third day after operation .3 per cent. (.7 gm.)  
 Salt excretion thirteenth day after operation .26 per cent. (1.1 gm.)  
 Albumin present for one day. No casts seen. Animal killed on the fourteenth day.

RABBIT 3.—Weight 1,850 gm. Weight of removed kidney 7 gm. Weight of remaining kidney after death 7 gm.

Sulphonephthalein excretion first day after operation 25 per cent.  
 Sulphonephthalein excretion second day after operation 30 per cent.  
 Sulphonephthalein excretion fifth day after operation 55 per cent.  
 Sulphonephthalein excretion twentieth day after operation 70 per cent.  
 Lactose excretion first day after operation 5 hours.  
 Lactose excretion nineteenth day after operation 5 hours.  
 Iodid excretion first day after operation 24 hours.  
 Iodid excretion nineteenth day after operation 24 hours.  
 Salt excretion third day after operation .6 per cent.  
 Salt excretion nineteenth day after operation 1.1 per cent. (1.5 gm.)

Albumin present for one day. Hematuria for one day. Animal killed on the twenty-first day.

Clamping of the renal circulation up to forty minutes, in the majority of cases, produced a definite disturbance in renal function. Its intensity bore no relation to the length of time the vessels were clamped, nor was the vascular or tubular function chiefly affected (Protocols I to IV). This was shown by the presence of albumin and casts in the urine, by a diminished phthalein output of varying degree, and by a delayed lactose and iodid excretion. Salt was constantly well excreted. The animals recovering, regained nearly normal function within six days, showing that the disturbance was slight and temporary. One animal failed to return to a normal phthalein output. No explanation was found for this at autopsy. Two animals with circulation clamped for forty minutes died quickly with marked signs of renal insufficiency.

All the animals, however, with one kidney removed and the circulation of the remaining kidney clamped for an hour (Protocol V) died within eight days. There was evidence clinically as well as by these tests of extreme disturbance of function. An interesting proof of how important a part in renal surgery the unoperated kidney plays is seen in Protocol VI. In this series, though the circulation was clamped for an hour, with a normal kidney remaining, the function was but slightly and temporarily disturbed.

That the effect of nephrectomy alone did not produce the results obtained is seen from Protocol VII. In two animals the functional tests were unaffected. In the third the phthalein output was slightly reduced for three days. The other tests were normal.

Pathologically, similar changes were noted to those described by previous authors. The earliest changes, grossly, were marked congestion with scattered minute hemorrhages. As the length of time following



the operation increased, the kidneys hypertrophied, but otherwise appeared normal.

Microscopically first were seen hemorrhage, edema, destruction of cells, as evidenced by poor staining, the presence of hyaline-like casts in the tubules, and foci of leukocytes, endothelial phagocytes and mononuclear cells between the tubules and around the glomeruli. In time the kidney regained nearly normal appearance. In a few kidneys there were areas of increased interstitial tissue, and rarely thickening of a few glomerular capsules. It was not possible to find any definite relationship between the anatomical changes and the disturbance of function, except that in those kidneys clamped for an hour there was more edema and necrosis than in the others. In none of the kidneys examined was there evidence of any progressive lesion. The changes were all acute or healed.

From these experiments on animals it seems clear that renal circulation may be clamped for at least forty minutes without danger of marked permanent damage to the kidney either histologically or functionally. How far these results are applicable to human surgery is an open question. From the fact that these experiments were made under the most disadvantageous conditions possible, that is, with only one functioning kidney, it would seem fair to assume that in man with one kidney normal, the circulation of the other kidney could be clamped for a considerable length of time without harm.

#### CONCLUSIONS

1. In rabbits and dogs with one kidney removed, the circulation of the other kidney may be clamped for as long a time as forty minutes with recovery. If the renal circulation is clamped for a longer time, the animals die with signs of renal insufficiency.

2. In animals with the circulation clamped for not longer than forty minutes, temporary disturbance in renal function is produced as shown by the presence of albumin and casts in the urine, by a diminished phthalein output and by a delayed lactose and iodid excretion. Normal function is regained within six days.

3. Acute or healed pathological changes are found in kidneys so treated. The acute changes consist of edema, hemorrhage, necrosis and cellular infiltration. The healed changes consist in foci of connective tissue. No progressive lesion is found.

4. Except in the most extreme cases, there is no definite relation demonstrable by the functional tests used between the pathological and functional disturbances produced.

5. In rabbits with one normal kidney, the circulation of the other kidney may be clamped for at least an hour without permanent injury to the animal's general condition or renal function.

OBSERVATIONS ON THE PATHOLOGICAL CHANGES IN  
THE THYROID GLAND IN A CRETINISTIC  
VARIETY OF CHONDRODYS-  
TROPHIA FOETALIS \*

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That the lesion variously known as chondrodystrophia foetalis, achondroplasia, fetal rickets and the like, is of great antiquity is attested by the numerous instances in which the disease finds representation among pottery and statuary of the ancients (Parrot,<sup>1</sup> Pierre Marie,<sup>2</sup> Porak and Durante,<sup>3</sup> Regnault,<sup>4</sup> Charcot and Richie,<sup>5</sup> Rischbieth<sup>6</sup> and others). The models of the Egyptian gods, Ptah and Bes, to be found in the Louvre, the existing statuette in caricature of the Roman Emperor Caracalla, the figures of the gladiator dwarfs in the service of the Emperor Domitian, as well as the subjects of certain paintings, notably by Velasquez, among them that of Sebastiano de Moro, a dwarf of the Spanish Court, are all recognizable examples of fetal chondrodystrophy.

Historical interest attaches to the statement that, in the sixteenth century, Catherine de Medici revived the ancient custom of according gnome-like creatures a position in the social firmament of the Court, where they enjoyed unusual latitude of speech and action in the capacity of jesters, and emulating the Emperor Heliogabalus, she sought even further to gratify her perverted taste by celebrating marriages between dwarfs with the purpose of perpetuating a stunted race comparable to the ethnic dwarfs of fact and fable. A survival of the same perversion consists in exploiting the chondrodystrophic dwarf in the circus of to-day that his deformed body may be held up to gibe, ridicule or vulgar curiosity. On the other hand, numbers of characters distinguished by action or intellect are known or are reputed to have been the victims of fetal chondrodystrophy, among them the author of Æsop's Fables;

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\*Submitted for publication April 5, 1913.

1. Parrot: Bull. Soc. d'anthropolog. de Paris, Series 3, 1878, i, 296.

2. Marie, Pierre: Presse méd. 1900, iv, 12.

3. Porak and Durante: Nouvelle Inconographie de la Salpêtrière, 1905, xviii, 481.

4. Regnault: Arch. gén. de méd. (new series), 1902, vii, 232.

5. Charcot and Richie: Des difformes et les malades dans l'ast, Paris, 1889, p. 12.

6. Rischbieth: Eugenics Laboratory Memoirs, xv, Parts vii and viii, Section xva, p. 355, London, 1912.

Philetas of Cos, tutor of Ptolemy Philadelphus and a poet and grammarian of renown; Licinius Calvus, a rhetorician; Atilla, King of the Huns; Wladislaus, called "Cubitalis," King of Poland, and noted for intelligence and military sagacity, and others of like account.

From an etiological standpoint chondrodystrophia foetalis is scarcely less obscure than it was in the beginning and of the several factors which have been invoked to explain its origin none appears to be worthy of serious consideration other than that the condition is always congenital, completing its development in the first few weeks of intra-uterine existence, that it preponderates in the female sex (Kassowitz), and that it sometimes presents familial characteristics. In the latter connection Rischbieth,<sup>6</sup> who has contributed an admirable paper to the eugenics of dwarfism, states that, while a chondrodystrophic parent usually begets normal children, heredity not uncommonly displays itself in the shape of a deformed progeny. The pathogenesis is no less conjectural for having been referred to various types of intoxication, including infections and placental and glandular perversions. In the latter connection Abels<sup>7</sup> quotes a suggestive instance in which a chondrodystrophic infant was born of a mother who, before and during pregnancy, had accustomed herself to enormous quantities of thyroid extract. Hofmeister,<sup>8</sup> on the contrary, states that extirpation of the thyroid gland from young rabbits is followed by cessation of growth in the bones and by changes which are comparable to those encountered in chondrodystrophia foetalis.

The first really adequate contribution to the pathology of chondrodystrophia foetalis was made in 1860 by Mueller,<sup>9</sup> who observed the disease in certain forms of cattle and identified the changes with those previously noted by Virchow<sup>10</sup> in the human skeleton. Mueller's observations have since been extended to include certain short-limbed domestic animals, more particularly the dachshund, bull dog, Pekinese spaniel, and Aberdeen terrier, but observation has shown that the likeness between them and the subjects of chondrodystrophia foetalis is fancied rather than real and that these animals merely typify combinations of different racial peculiarities brought about by artificial selection.

It was reserved for Kaufmann,<sup>11</sup> in 1892, to establish a classification based on anatomical and histological studies in human beings which showed that chondrodystrophia foetalis is referable to disturbances in the primordial cartilage cells manifested by derangement of the columnar

7. Abels: Kassowitz' Festschrift, Berlin, 1912, p. 1, quoting Cavazanni, *Pediatrica*, 1907.

8. Hofmeister: Quoted by Kaufmann, *Spezielle path. Anat.*, 1911, ii, Part 6, p. 733.

9. Mueller: *Wurzb. med. Zeit.*, 1860, i.

10. Virchow: *Gesammte Abhandlung.*, 1852.

11. Kaufmann: *Untersuchungen u. die sogenannte foetale Rachitis*, 1892; *Spezielle path. Anat.*, 1911, ii, Part 6, p. 733.

formation which normally precedes ossification. Depending on the extent of these cellular variations in cartilage, Kaufmann differentiated three anatomical types, all of which may be found in the same individual, although, as a rule, one of them is apt to dominate. In the first type — chondrodystrophia foetalis hypoplastica — proliferation of cartilage cells is inhibited, while the second variety — chondrodystrophia foetalis hyperplastica — is characterized by cartilaginous overgrowth. In both of these forms the intercellular substance is well preserved. In a third type — chondrodystrophia foetalis malacica — the cartilage is soft, the intercellular substance is gelatinous and centers of ossification are rudimentary or absent. In addition to these cartilage changes endochondral ossification is prematurely suspended, while periosteal osteogenesis continues, so that several factors combine to interfere with or to modify the growth of certain bones and to produce a clinical picture which is at once characteristic and scarcely to be mistaken for that of any other known disease.

Individuals of the chondrodystrophic type, the majority of whom are still-born or die young, are deformed in certain bones which are conceived in cartilage and whose scheme of development is completed before the eleventh week of embryonal life;<sup>12</sup> that is to say, the osseous complements of the base of the skull and the pelvis, the ribs and the long bones of the extremities. Consequently the child at birth is greatly abbreviated in stature, the head is large, and the extremities, which may be crooked, are symmetrically shortened and thickened, varying between micromelia and phocomelia, and the cartilaginous termini are enlarged and nodular. The finger-tips, which normally reach to the level of the upper third of the thigh, rest at the crest of the ilium or shortly below it. The hands are broad and pudgy and the middle digit is shortened. The fingers are roughly conical in outline and separate in pairs, combining with the out-stretched thumb to form the "*main en trident*" of the French. The face is relatively small, the nose depressed at the root and the frontal eminences are exaggerated. These features unite with the large cranial vault to lend a pyriform outline to the face. The trunk is usually well formed and of normal size, although the abdomen frequently is protuberant. The skin, which develops out of proportion to the growth of the bones, is thrown into folds, especially over the joints and corresponding to the normal creases. It is often waxy in appearance and the subdermal tissues may be edematous or excessively infiltrated by fat. In those who attain adult life these characteristics are preserved, with the exception of certain modifications referable to the skin, but as development proceeds the individual is endowed with muscular strength, sexual vigor and a degree of intelligence often noticeably in excess of the average. In addition to alterations in the bony skeleton, certain

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12. Consult Mall: *Am. Jour. Anat.*, 1905-6, v, 433.



numbers of chondrodystrophic individuals present external signs that are suggestive of cretinism; so much so, indeed, that some observers have proclaimed that chondrodystrophia foetalis and infantile myxedema are manifestations of the same fundamental disorder, or, at all events, that the two conditions are closely related. Kaufmann and his adherents deny such relationship. While admitting that there is a form of chondrodystrophia foetalis in which the physiognomy is cretinoid in type, they affirm that it is due almost entirely to premature synostosis of the os tribasillare and that it is an integral part of the chondrodystrophic process, arising independently of alterations in the thyroid gland. It is furthermore pointed out that chondrodystrophic individuals sometimes reach adult life, in which event, in contra-distinction to cretins, they are intelligent, sexually capable and possessed of muscular strength.

The relationship of chondrodystrophia foetalis to cretinism was originally insisted on by Mueller<sup>9</sup> and by Eberth,<sup>13</sup> both of whom pointed out that the so-called "bull dog calf" presents cretinoid appearances. Some years later Leblanc<sup>14</sup> noted the association of chondrodystrophia foetalis and myxedema in calves and attempted to implicate the thyroid gland as the causative factor in the production of both the skeletal and myxomatous changes. He based this conclusion on a single observation of "arrested development" in the thyroid! Histological examination was not made. It remained for Seligmann<sup>15</sup> to demonstrate tangible changes in the thyroid gland of the "bull dog calf" and to establish the coexistence, in cattle at least, of chondrodystrophia foetalis and congenital myxedema. Seligmann examined specimens of Dexter calves born in Kerry. In all of them the skin was thick and coarse and the subcutaneous tissues were myxomatous. The head was large and brachycephalic, the forehead bulging and the nose depressed. The tongue was large and protruded from the half-open mouth. All the limbs were extremely reduced in length and their bones were stunted and provided with thickened, deformed, softened cartilages.\* In every instance the thyroid was irregularly lobulated and, on microscopic examination, the alveolar architecture was scarcely recognizable and the cells were arranged in clumps or branching columns. Colloid was practically absent and the gland was abnormally well vascularized.

As to the influence of the thyroid in the production or modification of chondrodystrophia foetalis in human beings, we have been able to find very little in the literature. Kaufmann, Legry and Regnault,<sup>16</sup> and others have submitted the thyroid to histological examination with nega-

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13. Eberth: Festschrift, Leipzig, 1878.

14. Leblanc: *Compt. rend. Soc. de biol.*, 1902, liv, 88.

15. Seligmann: *Trans. Path. Soc. London*, 1904, lv, 1.

16. Legry and Regnault: *Compt. rend. Soc. de biol.*, 1902, liv, 567.



tive results. In the same way Marie,<sup>2</sup> Cestan<sup>17</sup> and Christopher<sup>18</sup> have failed to note beneficial effects from the administration of thyroiodin and thyroid extract. Hektoen,<sup>19</sup> however, has recorded an instance of symmetrical micromelic dwarfism in a fairly intelligent man about 45 years of age, whose bony frame-work was the seat of a series of complex changes partaking of the nature of both chondrodystrophia foetalis and osteogenesis imperfecta. There were no demonstrable signs of cretinism. Nevertheless, the thyroid was almost completely transformed into fibrous tissue and was practically free from iodine. The weight of the pituitary body, however, was considerably in excess of the normal, in view of which Hektoen suggested that the effects of fibrous replacement of the thyroid had been compensated by increased secretory activity on the part of the hypophysis.

We have recently had occasion to study the anatomical peculiarities in five subjects of fetal chondrodystrophy—four times in infants and once in an adult—and have observed changes which, we believe, serve to establish the fact that a certain proportion of cases of chondrodystrophia foetalis in human beings is intimately associated with cretinism. Moreover, in every case we have been able to demonstrate histological alterations in the thyroid gland which suffice to account for the cretinistic phenomena.

In preparing our cases for publication we have omitted all reference to the several clinical histories, none of which contains information of value in the present connection, and the autopsy protocols have been abbreviated as much as is consistent with an intelligible presentation.

#### AUTOPSY PROTOCOLS OF AUTHORS' CASES

CASE 1.—Autopsy 3415. Post mortem examination by Drs. Wallace and Symmers.

The body was that of a female child, 23 months of age, 70 cm. in length. The stature was markedly abbreviated, squatty in type and the body presented a bloated appearance. The skin was tense, glistening and waxy, and the subdermal tissues were the seat of firm edema, most noticeable over the dorsum of the feet and in the region of the external genitalia. The head was markedly increased in size and dolichocephalic. It was 45 cm. in circumference at the level of the occipital protuberance. The eyes were widely separated and the eyelids, cheeks, lips and wings of the nostrils were greatly thickened. The abdomen was protuberant. The arms were shortened, measuring 26 cm. in length, the hypothenar eminences resting at the level of the crest of the ilium. The legs, which were large in caliber, measured 32 cm. in length. On section the subcutaneous tissues of the thoracic and abdominal walls presented the appearance of gelatinous edema. The loose tissues in the anterior mediastinum and around the kidneys were similarly involved. On stripping the muscles from the anterior thoracic wall a well-marked rosary came into view. This, on dissection, was found to consist of hyperplastic cartilages which overlapped the anterior extremi-

17. Cestan: *Nouvelle Iconographie de la Salpêtrière*, 1901, xiv, 277.

18. Christopher: *American Med.*, June 7, 1902.

19. Hektoen: *Am. Jour. Med. Sc.*, new series, 1903, cxxv, 751.

ties of the ribs and were specially conspicuous on the inner aspect of the chest. The foramen magnum was distinctly diminished in its antero-posterior diameter. The pelvis was contracted in all diameters and its bony elements were thickened. The long bones of both the upper and lower extremities were greatly shortened, the shafts were thickened and firm and the cartilages were enlarged and nodular. Thyroid: The thyroid weighed 4.4 gm. The left lobe was one-third again as large as the right. The gland was pinkish in color and firm in consistence. Pituitary: This organ was noticeably diminished in size. Lymphoid System: The lingual and faucial tonsils, the lymphoid follicles in the spleen and gastrointestinal tract, including Peyer's patches, and the mesenteric lymph-nodes were markedly hyperplastic. Adrenals: Both adrenals were small and their cortices were narrowed. Aorta: This vessel was distinctly hypoplastic.

Anatomical Diagnosis: Chondrodystrophia foetalis; myxedema; chronic interstitial thyroiditis; status lymphaticus with concomitant hypoplasia of the adrenal cortex and of the aorta; bilateral hydrothorax, congestion and atelectasis of lungs; hypoplasia of pituitary.

Histological Examination of Thyroid: On histological examination of the thyroid it was found that the normal architecture was scarcely recognizable. The gland was traversed at numerous intervals by bands of dense, richly vascularized connective tissue intercommunicating in such a way as to divide the parenchyma into solid islands of varying shapes and sizes, the individual cells being closely packed. The parenchyma cells were pale and their cytoplasm was finely granular, while the nuclei varied in size and chromatic richness. In rare instances an alveolus was encountered which was comparable to the normal; that is to say, it was lined by a single layer of cuboidal epithelium, but colloid was practically absent. Lying in the connective tissue at scattered intervals were clumps of richly chromatic spindle-shaped cells. In places these cells did not confine themselves to the interstitial framework, but encroached on and finally replaced the parenchymatous islands to such an extent that a low power field not infrequently was found to contain from ten to thirty alveoli in which the epithelium had been replaced by clumps of spindle cells standing out in strong contrast to the epithelial islands.

CASE 2.—Autopsy 3484. Section limited to abdominal incision. Post mortem examination by Dr. Symmers.

The body was that of a female child, 5 months of age, 53 cm. in length. The skin was pale and irregularly thrown into folds. The head was markedly enlarged and dolicocephalic. The upper eye-lids on both sides were puffy, giving a frog-like expression to the face. The nose was large, depressed at the root, and the wings of the nostrils, lips and cheeks were distinctly thickened. Both arms were shortened, the tips of the fingers reaching slightly below the level of the crest of the ilium. The abdomen was decidedly protuberant. On opening the abdomen a large amount of mucoid material of the appearance and consistence of Wharton's jelly was found in the loose connective tissue of the mesentery and of the gastro-hepatic omentum. Tongue: The tongue was enlarged and projected slightly beyond the lips. Thyroid: The thyroid measured 2.5 x 1.5 cm. and was coarsely lobulated. The organ was pinkish in color, the cut surface was flesh tinted and scattered over it were numerous minute grayish strands. No colloid was apparent to the naked eye. Lymphoid System: The lymphoid tissues were atrophic throughout.

Anatomical Diagnosis: Chondrodystrophia foetalis; cretinism; chronic interstitial thyroiditis; macroglossia; inanition.

Histological Examination of Thyroid: On examination of the thyroid gland it was practically impossible to identify the tissue from its microscopic appearances. The parenchyma was divided into large, variously shaped lobules by broad bands of coarsely fibrillated, poorly nucleated connective tissue from which delicate strands penetrated the cell groups at frequent intervals and subdivided them into small alveoli. The alveoli were crowded with large, flattened,

indistinctly outlined cells, the nuclei of which varied greatly in size and chromatic content, while the cytoplasm was scanty and finely granular. The majority of the nuclei were large, rounded and vesicular in appearance. Others were small and deeply staining. Of the nuclear changes, however, the most striking consisted in the great profusion of cells with huge, rounded, oval, or occasionally indented, nuclei which were rich in chromatin. In rarer instances these giant nuclei were vesicular in type. Mitotic figures were not found, colloid-containing vesicles were absent and the supporting tissues were poorly vascularized. In no instance did the parenchyma cells display any tendency to infiltrate the connective tissues at the periphery of the lobules.

CASE 3.—Autopsy 3503. Post mortem examination by Dr. Hagemeyer.

The body was that of a male child, 15 months of age, 60.5 cm. in length. The head was markedly enlarged, measuring 46 cm. in circumference at the level of the parietal eminences, and the anterior fontanel was open and measured

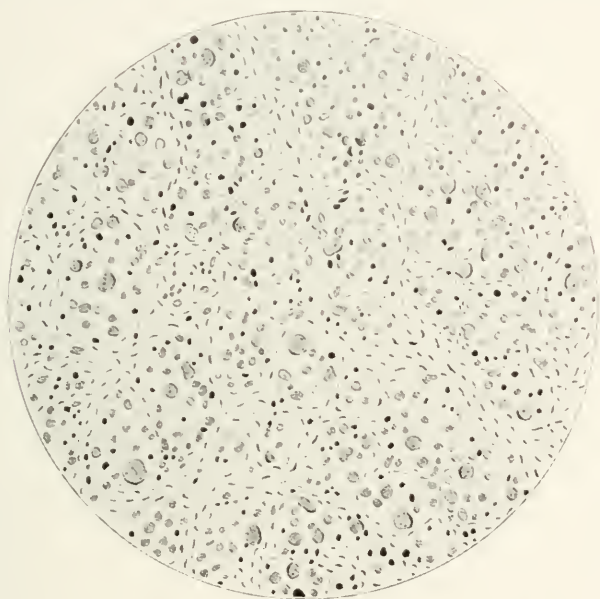


Fig. 1.—Case 2.—High power drawing of thyroid showing the presence of alveoli surrounded by hyperplastic connective tissue and partially filled by epithelial cells of variable size and chromatic richness. Drawn with Edinger's apparatus.

5 x 3 cm. The ears were large and stood out prominently and the bridge of the nose was sunken. The arms were greatly shortened, measuring 30 cm. in length. The legs were pudgy and measured 20 cm. in length and the feet were broad. The abdomen was protuberant, measuring 38 cm. in circumference at the level of the umbilicus. There was a deep anterior curvature in the sacral region and a well formed thoracic rosary. Bony Skeleton: The anterior ends of the ribs were coarsely beaded, especially on the inner aspect. The sacrum projected into the pelvis to such an extent that the superior opening permitted the entrance of one finger only, while the lateral diameter corresponded to the breadth of two fingers. The foramen magnum was normal in size, but presented a tubercle springing from the posterior border into the spinal canal. Brain: The pia mater covering the upper surface of the cerebral hemispheres was thickened and gelatinous. Thyroid: The thyroid was described as "slightly enlarged, but otherwise

normal." Lymphoid System: This system presented the changes incident to well marked status lymphaticus associated with characteristic narrowing of the suprarenal cortices.

Anatomical Diagnosis: Chondrodystrophia foetalis; myxomatous transformation of the pia arachnoid; status lymphaticus.

Microscopic Examination of the Thyroid: Histological investigation of the thyroid revealed the presence of excessive over-growth of interstitial connective tissue, the trabeculae of which were disposed in such fashion as to divide the substance of the gland into irregular islands and to separate the individual



Fig. 2.—Case 4.—X-ray photograph of chondrodystrophic child, 2 months of age, 42 cm. in length, showing characteristic cartilaginous and bony changes. Note the enlarged cranial vault, the depressed nose, hyperplasia of the costochondral cartilages and those of the long bones, diminution in length and increase in thickness of the shafts of the bones of the extremities and the contracted pelvis.

alveoli one from the other. The connective tissue was moderately cellular and fairly well vascularized. For the greater part the alveoli were well formed and filled by colloid and varied in size within normal limits. They were lined by a single layer of well nucleated cuboidal epithelium. In occasional instances



groups of alveoli occurred the individuals of which were markedly compressed by connective tissue over-growth and were filled by small oval or spindle cells with densely chromatic nuclei.

CASE 4.—Autopsy 3055.<sup>20</sup> Post mortem examination by Dr. Norris.

The body was that of a female child, 2 months of age, 42 cm. in length. The head was large and measured 43 cm. in circumference. Both fontanelles were open, the anterior measuring 5 x 6 cm. The eyes were widely separated and bulging and the lids were markedly thickened and corrugated. The tongue was large and protruded between the lips which, with the wings of the nostrils and the soft tissues of the ears, cheeks, forehead, neck and vulvae, were thickened. The arms were greatly shortened, measuring 15 cm. in length. The legs measured 20 cm. in length, the soft tissues of the dorsum of the feet were thickened and the toes were stubby. The skin of the forehead and of the upper thighs was thrown into coarse folds. The abdomen was markedly protuberant. On section the anterior chest wall exhibited a distinct rosary corresponding to hyper-

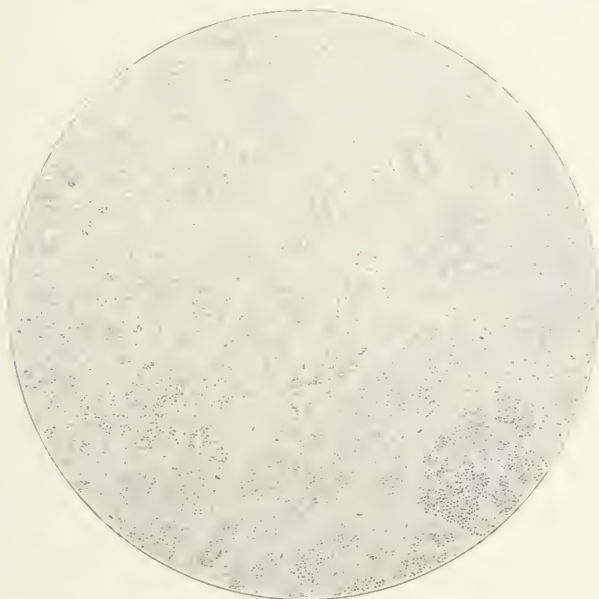


Fig. 3.—Case 4. Low power drawing of thyroid showing interstitial overgrowth and irregularly shaped alveoli filled by densely chromatic cells. Drawn with Edinger's apparatus.

plastic costal cartilages which were most conspicuous on the pleural side. The foramen magnum was very narrow in its anteroposterior diameter, the shafts of the long bones were short and thick and their cartilages were hyperplastic. Thyroid: The thyroid was very small, the lobes were symmetrical and weighed together 1 gm.

Anatomical Diagnosis: Chondrodystrophia foetalis; cretinism; atrophy of thyroid gland; atrophy of lymphoid system.

Histological Examination of Thyroid: On microscopic examination of the thyroid it was found that the normal architecture had been extensively altered and that the tissue could not be identified except for the presence of scattered groups of empty vesicles lined by very low cuboidal epithelium. The interstitial

20. For previous record of this case see La Fétra, *Am. Jour. Dis. Child.*, 1913, v, 18.



tissues were markedly thickened and supported innumerable irregularly outlined cellular islands the individual constituents of which were densely packed and were provided with small, rounded or cylindrical, richly chromatic nuclei.

**Skin:** Microscopic examination of the skin showed marked atrophy of the epithelial papillae. The subdermal structures were greatly thickened and edematous and made up of coarse, swollen, hyalin fibers in which the nuclei were very small, few in number and poor in chromatin. In the deeper parts fat lobules occurred in abundance.

**Muscle Tissue:** Microscopic examination of the peripheral muscles revealed the existence of marked atrophy of the individual fibers with actual replacement at scattered intervals by clumps of cells with spindle shaped nuclei.

CASE 5.—Autopsy 3445. Post mortem examination by Dr. Norris.

The body was that of a female, 31 years of age, 116 cm. in length. The head was large, brachycephalic, and measured 55 cm. in circumference. The nose was small, short and depressed at the bridge. The arms were abbreviated, measuring 38 cm. in length and the hands, which were small, did not reach beyond the crest of the ilium. The legs measured 43.5 cm. in length. The abdomen was protuberant and the subcutaneous fat was very abundant. Further examination of the body revealed nothing worthy of mention other than characteristic hyperplastic changes in the cartilages of the long bones, thickening and sclerosis of the shafts and diminution in the anteroposterior diameter of the foramen magnum. The thyroid weighed 6.5 gm. and was deep red in color.

**Anatomical Diagnosis:** Chondrodystrophia foetalis; chronic parenchymatous nephritis; fatty degeneration of the heart and liver; acute pharyngitis; fibromyoma of uterus; sclerosis of ovaries; congestion of thyroid.

**Histological Examination of Thyroid:** On microscopic examination of the thyroid it was found that the parenchyma was irregularly divided into large islands by broad bands of coarsely fibrillar, poorly nucleated connective tissue. Delicate subsidiary trabeculae penetrated the islands and surrounded the individual alveoli. In the connective tissue framework deeply injected vessels were numerous. In the larger trabeculae the vessels were of considerable size, while the more delicate strands around the alveoli supported a vascular net-work the ramifications of which included practically every alveolus in a capillary loop, so that the contents of the vessels were separated from the epithelium of the alveoli by a single layer of endothelium. The alveoli varied in size within moderate limits. The smallest were free from colloid and scarcely larger than those encountered in the fetal gland, while the largest approximated in size the alveoli of the adult thyroid and were partially or completely filled by faintly staining colloid. Although the smaller alveoli predominated, the larger, colloid-containing vesicles were present in considerable numbers. The epithelial cells in the alveoli were fairly large, oval or rounded, and were provided with moderately chromatic nuclei and finely granular, faintly acidophilic cytoplasm. The larger alveoli were lined by a single layer of cells, while the smaller were partially or completely filled by detached epithelium. In addition to these changes there occurred in the connective tissue at scattered intervals small foci of cells with richly chromatic, spindle-shaped nuclei and scarcely perceptible cytoplasm. In no instance was it possible to detect any sign of invasion of the alveoli by these cells.

#### CONCLUSIONS

1. There is a form of chondrodystrophia foetalis in which, in addition to changes in the osseous system, we feel justified in emphasizing the occurrence of modifications in the soft tissues which are attributable to pathological defects in the thyroid gland. The modifications in question consist of thickening of the lips, cheeks and eyelids, the wings of the

nostrils and the lobes of the ears, macroglossia, hypertrophic vulvae and myxomatous transformation of the subcutaneous and certain deep connective tissues, all of which, when combined with the large head and frog-like expression, the protuberant abdomen, prominent skin folds and pudgy extremities, fulfill the essential requirements for the diagnosis of cretinism. In the latter connection it is to be observed that the chondrodystrophic individual very often bears the so-called "cretinoid physiognomy" without presenting any evidence of cretinism. In these circumstances the large head, depressed nose and dwarfed body, while constituting a superficial resemblance to cretinism are, in reality, the outward expression of a disease which is referable to the bony system and which arises independently of demonstrable changes in the thyroid gland. To

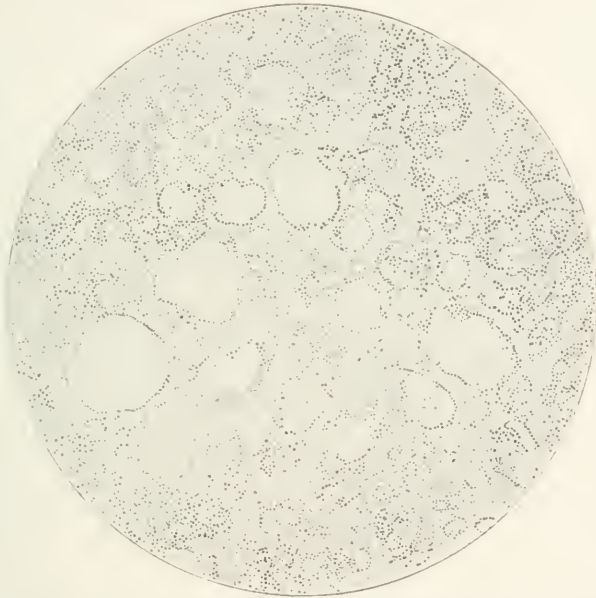


Fig. 4.—Case 5. Low power drawing of thyroid showing extensive connective tissue hyperplasia, alveoli partially or completely filled by detached epithelium, and colloid-containing vesicles. Female, aged 31. Drawn with Edinger's apparatus.

what extent, if any, functional disturbances in the developing thyroid are responsible for the skeletal deformities is an open question. When chondrodystrophia foetalis and cretinism coexist the picture becomes still further involved, and it may not always be apparent to what extent the physiognomy is determined by cretinism and how much of it is due to the chondrodystrophic process acting through premature synostosis of the os tribasillare.

2. In the cretinistic variety of chondrodystrophia foetalis which we have just described, the pathological changes in the thyroid gland are

characterized by an extensive chronic productive inflammatory process eventuating in replacement of the colloid vesicles by means of over-growth of alveolar epithelium, or by invasion and substitution of the alveoli by connective tissue elements derived from the interstitium, aided, in both instances, by compression exerted from the outside by the contracting fibrous trabeculae.

3. In Case 5 we are confronted by an instance of chondrodystrophia foetalis in a female 31 years of age, whose thyroid gland showed the histological changes incident to chronic interstitial thyroiditis. While most of the vesicles were free from colloid, considerable numbers were filled or even slightly distended. Unfortunately we are unable to give any information concerning the colloid content of the pituitary body. At the post mortem examination it was noted that the physiognomy was "cretinoid" in type, but signs of myxedema were not detected. In the absence of a history of cretinism in childhood we must yield to the natural conclusion that, during life, the patient's thyroid, although extensively diseased, was sufficiently active to protect her against myxedema.

4. In Case 3 the subject was a child, 15 months of age, in whose bony skeleton the signs of fetal chondrodystrophy were indisputable, but the changes indicative of cretinism were limited to myxomatous transformation of the pia-arachnoid and to enlargement of the ears, pudgy legs and spade-like hands. The thyroid was described as "slightly enlarged," and histological examination showed evidences of an extensive chronic interstitial thyroiditis in which, however, colloid-containing vesicles occurred in large numbers. Here, again, we must conclude that the child was, to an extent, protected against myxedema as the result of partial preservation of the functional activity of the thyroid gland.

In conclusion, we wish to acknowledge our gratitude to Dr. Charles Norris for his kindly interest and cooperation, and to thank Dr. K. D. Bryson for aid in the preparation of the illustrations. Acknowledgement is also due Drs. La Fétra and Lindeman.

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STUDY XVII: EXPERIMENTAL CHRONIC NEPHRITIS PRO-  
DUCED BY THE COMBINATION OF CHEMICAL  
(URANIUM NITRATE) AND BACTERIA  
(*B. COLI COMMUNIS*)\*

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This study has been made at the suggestion of Dr. Henry A. Christian in an attempt to produce a chronic nephritis in animals in a manner comparable to that used by Opie<sup>1</sup> in the production of an experimental cirrhosis of the liver. Opie found that repeated injections of chloroform, which causes a necrosis of the liver, and *Bacillus coli communis*, whose relation to the liver is quite intimate, produced a well-marked sclerosis of the liver. In the present experiments repeated injections of uranium nitrate and *B. coli communis* were made in rabbits to see if in similar way a sclerosis of the kidney would be produced. Uranium nitrate is known to produce an acute nephritis similar to that occurring in human beings in which there is very extensive necrosis of the tubular epithelium, and has been shown by Dickson,<sup>2</sup> Smith<sup>3</sup> and others, to produce a chronic nephritis when injected repeatedly over a long period of time. Although the relationship between *B. coli communis* and the kidney is not as intimate as that with the liver, infection of the urinary tract with colon bacilli is fairly common and it has been suggested that these bacteria may play a part in the chronic nephritis occurring in man.

Rabbits were the animals used. Although full grown rabbits were selected in most of the experiments, it was found very difficult to keep them alive long enough to produce any chronic lesion. In fact, about one-half of all the rabbits started, died within a few days, either from a septicemia from *B. coli communis* or from the uranium nitrate, or from both. Only eighteen out of forty rabbits used, survived long enough to produce any chronic lesions. These eighteen were carried through a

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\* A series of studies on experimental cardiovascular disease has been published previously; see Study XVI. THE ARCHIVES OF INT. MED., 1913, xi, 517, for reference to these.

\* From the Laboratory of the Department of the Theory and Practice of Physic, Medical School, Harvard University.

\* This work was done under a grant from the Proctor Fund for the Study of Chronic Diseases.

\* Submitted for publication March 25, 1913.

1. Opie: Jour. Exper. Med., 1909, xii, 237.

2. Dickson: THE ARCHIVES INT. MED., 1909, iii, 375.

3. Smith: THE ARCHIVES INT. MED., 1911, viii, 481.



period of life varying from a few weeks to five months. Some died, most of them were killed.

The exact method employed varied with the individual animal and can be found in the protocols. One was given 5 mg. of uranium nitrate subcutaneously every three weeks, and at the same time 0.25 c.c. of a twenty-four hour bouillon culture of *B. coli communis* intravenously every three days. Four were given 2 mg. uranium nitrate at intervals of three days for three doses, followed by three doses of 0.25 c.c. of a twenty-four hour bouillon culture of *B. coli communis* at three-day intervals, etc. The rest were given alternate doses of uranium nitrate 2 mg., and *B. coli communis* 0.25 c.c. at three-day intervals. In one



Fig. 1.—Rabbit 370. Magnification 65 diameters. Kidney showing area of connective tissue increase radiating into the cortex from the surface.

case, Rabbit 370, the dose of *B. coli communis* was increased during the last two months to 0.35 c.c.

The results of this treatment were that twelve out of the eighteen rabbits showed a moderately marked increased of the connective tissue, four a moderate increase, two a slight increase in the connective tissue. In thirteen the connective tissue was present in the outer cortex, usually starting from the surface in form of a scar running down one or more of the rays. (Fig. 1, Rabbit 370; Fig. 2, Rabbit 445; Fig. 3, Rabbit 461; Fig. 4, Rabbit 472.) In two of these cases the connective tissue was very marked in the outer cortex. In seventeen of the cases there was increased



fibrous tissue at the junction between the cortex and the medulla. (Fig. 5, Rabbit 471; Fig. 6, Rabbit 473; Fig. 7, Rabbit 460.) This seemed to be the favorite place for the greatest increase, as thirteen of the eighteen cases showed a marked increase in this region. Fourteen cases showed an increase of fibrous tissue in the medulla; in none was the increase very marked.

The connective tissue was quite fibrous in most cases, though in some it was fairly cellular. In most of the kidneys all three localities were involved, the connective tissue spreading from the surface down the rays in form of scars containing distorted glomeruli. These scars were con-

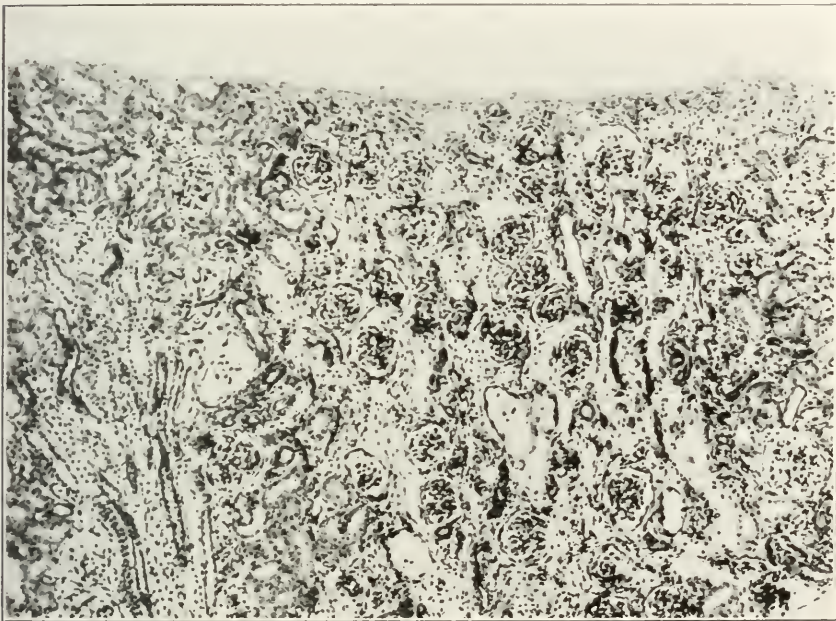


Fig. 2.—Rabbit 445. Magnification 65 diameters. Kidney showing area of connective tissue increase radiating into the cortex from the surface.

nected at the apex of the labyrinths by a thick band of connective tissue running parallel to the surface. The connective tissue in the medulla was never very marked.

Lymphocytic infiltration was present in ten of the eighteen cases, but was well marked in only three of the cases. No special part of the kidney was singled out, clumps of small lymphocytes being present here and there in all parts.

Most of the cases showed involvement of the glomeruli, this taking one or more of several forms. Ten showed distinct drawing together of the glomeruli by contraction of the connective tissue in the scars. (Figs.

1, 2 and 3.) Six showed a more or less marked dilatation of the capsule, usually accompanied by a shrinking of the tuft as from pressure of fluid in the capsular space. (Fig. 3.) Five showed shrinking of the glomeruli and twelve a distinct thickening of the capsular walls of many glomeruli. Ten showed also a thickening of the walls of the vessels in the tuft. Four showed a proliferation of the endothelial cells in the glomeruli and four an increased number of red blood-corpuscles in the glomeruli. Only seven out of the eighteen showed any dilatation of tubules such as Smith<sup>3</sup> speaks about. This is only well marked in two cases. (Fig. 8, Rabbit 442.)



Fig. 3.—Rabbit 461. Magnification 65 diameters. Kidney showing area of connective tissue increase radiating into the cortex from the surface.

In comparing the results of this series of experiments with those obtained by Smith<sup>3</sup> a general impression was obtained that chronic changes were more regularly encountered in the kidneys of rabbits which had received both uranium nitrate and *Bacillus coli communis* than in those rabbits which had received only uranium nitrate. It was found difficult, however, to make an actual comparison between the lesions in two sets, inasmuch as the rabbits of Smith's series of experiments varied considerably in the method of treatment from the rabbits in this series, both with regard to the dosage and the time elapsing between the last dose and the time of death.



There were, however, ten rabbits which were carried through an approximately equal period of active treatment. In five uranium nitrate alone was given; in the other five alternate doses of uranium nitrate and *Bacillus coli communis* were given, the total number of doses of the two being approximately the same as the total number of doses of uranium nitrate in the first five. In these rabbits the amount of connective tissue change showed no great preponderance in either set. On the whole, the connective tissue was rather more dense in the kidneys of the rabbits which had received both uranium nitrate and *Bacillus coli communis*. In addition to these ten rabbits, there were six rabbits, all of which had

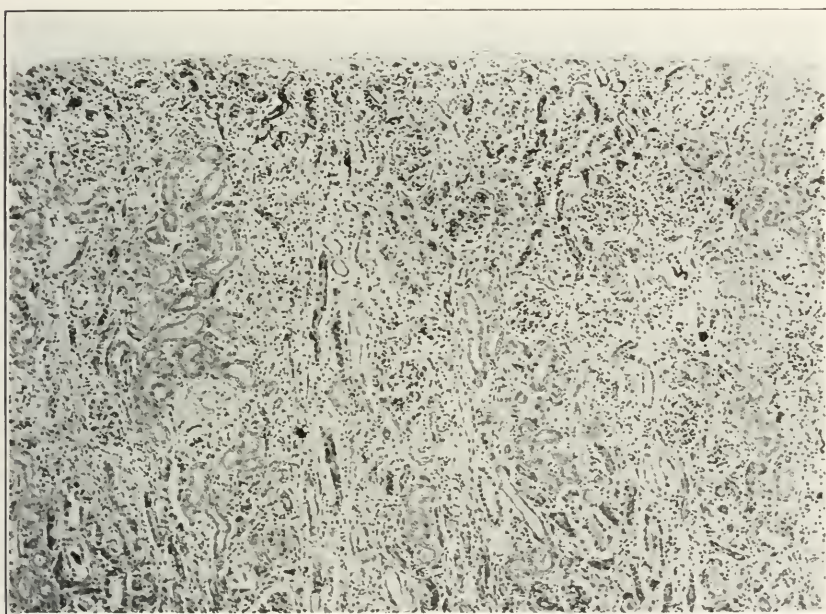


Fig. 4.—Rabbit 472. Magnification 65 diameters. Kidney showing area of connective tissue increase radiating into the cortex from the surface.

received approximately the same number of equal size doses of uranium nitrate over a period of equivalent time. Three of these in addition had received doses of *Bacillus coli communis*. In this set the rabbits that had received both uranium nitrate and *Bacillus coli communis* showed distinctly a more marked connective tissue change in their kidneys.

In any study of experimental chronic nephritis in the rabbit it must be kept in mind that the kidney of the rabbit very frequently shows a slight amount of connective tissue which has arisen under natural conditions in the animal, and is not the result of any form of experimental treatment. Occasionally quite marked chronic lesions are encountered

in these untreated animals. Making due allowance for these spontaneous lesions and utilizing as controls the tissues of the kidneys of those rabbits in the series which died of acute lesions very shortly after the beginning of the treatment, it would appear that the combination of uranium nitrate and *Bacillus coli communis* very definitely and quite regularly produces in the rabbit's kidney a chronic lesion with connective tissue increase, and other changes in many respects comparable to the lesions of chronic interstitial nephritis in man in so far as renal elements are concerned. The arteriosclerotic lesions that are so common in the human kidneys of chronic interstitial nephritis are not found in these rabbit kidneys.

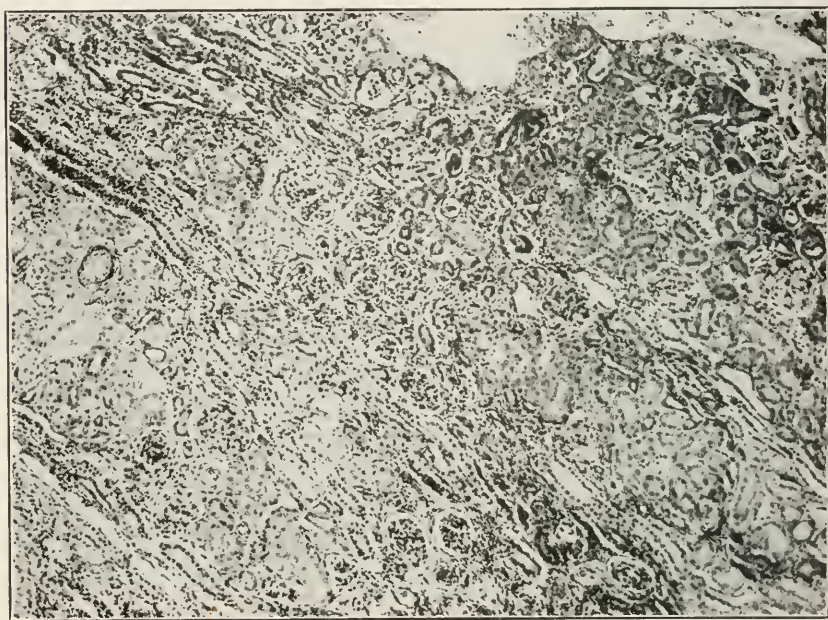


Fig. 5.—Rabbit 471. Magnification 65 diameters. Kidney showing connective tissue increase at the junction between cortex and medulla.

In the series of kidneys described in this paper it would seem fair to exclude those kidneys described as showing a moderate or slight increase in connective tissue change, inasmuch as these changes, though rather more frequent in this series, were not in excess of the lesions very commonly found in untreated rabbits. This, then, would leave twelve rabbits in which a considerable increase in connective tissue was produced in the kidney after injections of uranium nitrate and *Bacillus coli communis* covering a very considerable period of time as shown in the protocols. In other words, of 40 rabbits used in these experiments, 22 died so soon after the first injections as to exclude the possibility of any



chronic lesion; 18 lived long enough under treatment to show possible chronic lesions. Of these 18, 12 or two-thirds, showed a considerable degree of chronic change, sufficient to justify the term chronic experimental nephritis. The remaining 6, though showing some chronic change, are excluded from the series inasmuch as the change shown was not more than is frequently seen in rabbits not treated.

The protocols of the rabbits showing more definite changes are appended.

#### PROTOCOLS

RABBIT 366.—Weight, 1,650 grams. Uranium nitrate subcutaneously 3 mg., Dec. 4, 1911; 5 mg., December 9; 3 mg., December 11; 5 mg., December 13. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, December

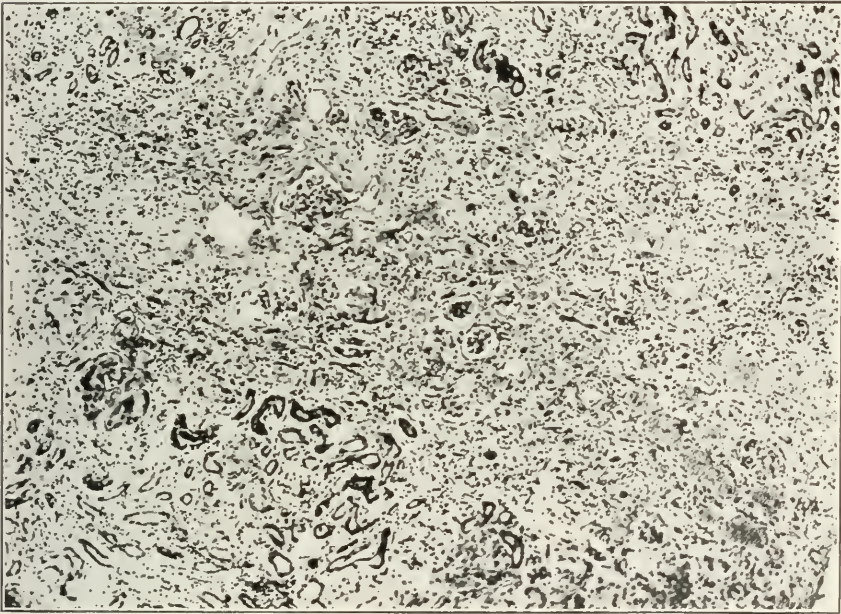


Fig. 6.—Rabbit 473. Magnification 65 diameters. Kidney showing connective tissue increase at the junction between cortex and medulla.

15, 18, 21. Uranium nitrate subcutaneously 2 mg., December 24, 27, 30. *B. coli communis* intravenously 0.25 c.c., Jan. 2, 5, 8, 1912. Uranium nitrate subcutaneously 2 mg., January 11, 14, 17. *B. coli communis* intravenously 0.25 c.c., January 20, 23, 26. Uranium nitrate subcutaneously 2 mg., January 29, February 1, 4. *B. coli communis* intravenously 0.25 c.c., February 7, 10, 13. Uranium nitrate 2 mg. subcutaneously, February 16, 19, 22. *B. coli communis* intravenously 0.35 c.c., February 25, 28, March 2. Uranium nitrate subcutaneously 2 mg., March 5, 7, 10.

Rabbit killed March 12, 1912. Duration of experiment seventy days. Kidney shows moderate connective tissue increase most marked in mid-zonal region.

RABBIT 370.—Caged Dec. 21, 1911. Urine negative. Uranium nitrate subcutaneously 2 mg., December 22, 24, 27, 30. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, Jan. 2, 5, 8, 1912. Uranium nitrate sub-



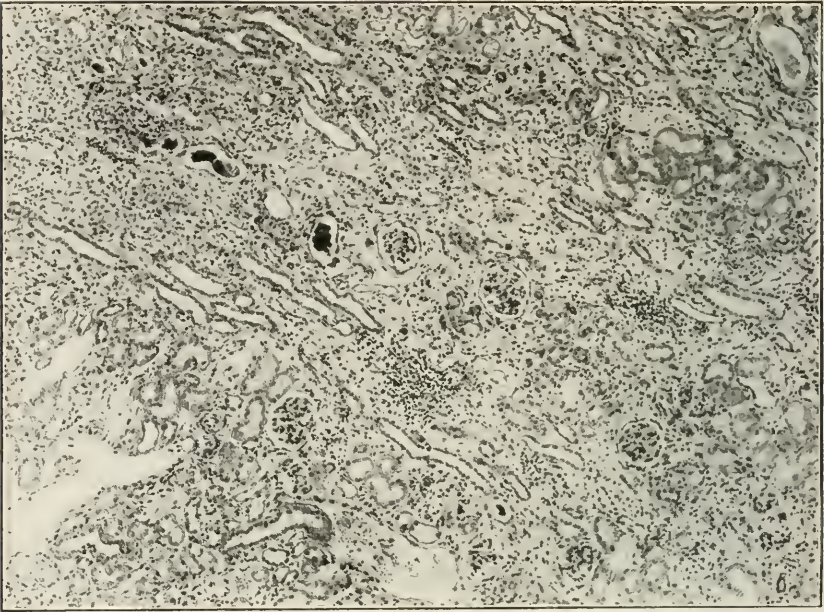


Fig. 7.—Rabbit 460. Magnification 65 diameters. Kidney showing connective tissue increase at the junction between cortex and medulla.

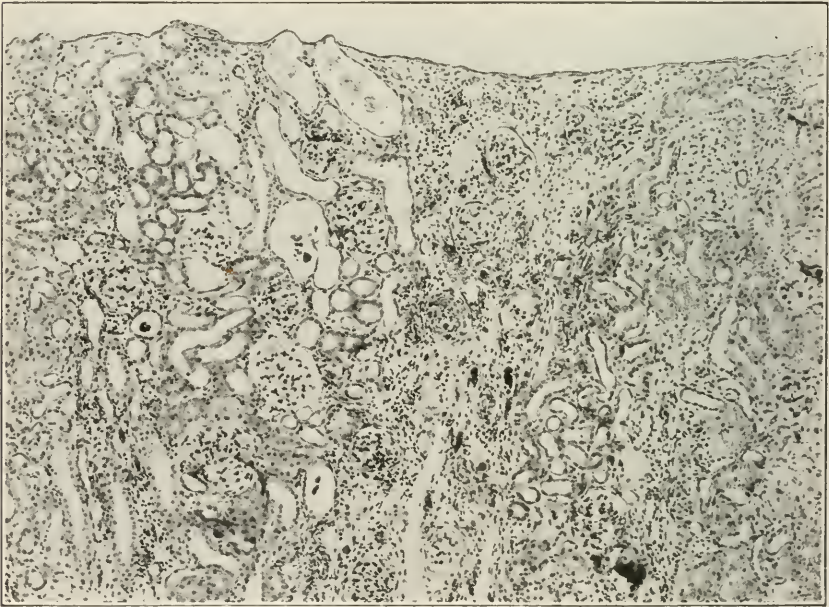


Fig. 8.—Rabbit 442. Magnification 65 diameters. Kidney showing connective tissue radiating into the cortex from the surface with dilatation of adjacent renal tubules.

cutaneously 2 mg., Jan. 11, 14, 17. *B. coli communis* intravenously 0.25 c.c., January 20, 23, 26. Uranium nitrate subcutaneously 2 mg., Jan. 29, February 1, 4. *B. coli communis* intravenously 0.25 c.c., February 7, 10, 13. Uranium nitrate subcutaneously 2 mg., February 16, 19, 22. *B. coli communis* intravenously 0.35 c.c., February 25, 28, March 2. Uranium nitrate subcutaneously 2 mg., March 5, 7, 10. *B. coli communis* intravenously 0.35 c.c., March 15, 18, 21. Uranium nitrate subcutaneously 2 mg., March 24, 27, 30. *B. coli communis* intravenously 0.35 c.c., April 2, 5, 8. Uranium nitrate subcutaneously 2 mg., April 11, 14, 17. *B. coli communis* intravenously 0.35 c.c., April 21.

Killed April 24, 1912. Duration of experiment 126 days. Kidney shows bands radiating in from the surface in which there is a moderate connective tissue increase, rather cellular in character.

RABBIT 382.—Weight, 1,400 grams. Caged Dec. 28, 1911. Urine negative. Uranium nitrate subcutaneously 3.33 mg. per K., December 30, 31. *B. coli communis* intravenously 0.25 c.c. of a 24-hour bouillon culture, January 27. Uranium nitrate subcutaneously 5 mg., February 17. *B. coli communis* intravenously 0.25 c.c., March 9. Uranium nitrate subcutaneously 5 mg. and *B. coli communis* intravenously 0.25 c.c., March 30. *B. coli communis* intravenously 0.25 c.c., April 2, 5, 8, 11, 14, 18. Uranium nitrate subcutaneously 5 mg. and *B. coli communis* intravenously 0.25 c.c., April 21. *B. coli communis* intravenously 0.25 c.c., April 24, 27, 30, May 4, 7, 10, 13, 15, 17, 20, 23, 26, 29, June 1. Uranium nitrate subcutaneously 5 mg. and *B. coli communis* intravenously 0.25 c.c., June 4. *B. coli communis* intravenously 0.25 c.c., June 7.

Killed June 10, 1912. Duration of experiment 165 days. Kidney shows condition quite similar to Rabbit 366.

RABBIT 440.—Caged March 3, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., March 24. *B. coli communis* intravenously 0.25 c.c., of 24-hour bouillon culture, March 27. Uranium nitrate subcutaneously 2 mg., March 30. *B. coli communis* intravenously 0.25 c.c., April 2. Uranium nitrate subcutaneously 2 mg., April 5. *B. coli communis* intravenously 0.25 c.c., April 14. Uranium nitrate subcutaneously 2 mg., April 18. *B. coli communis* intravenously 0.25 c.c., April 21. Uranium nitrate subcutaneously 2 mg., April 24. *B. coli communis* intravenously 0.25 c.c., April 27. Uranium nitrate subcutaneously 2 mg., April 30. *B. coli communis* intravenously 0.25 c.c., May 24. Uranium nitrate subcutaneously 2 mg., May 7. *B. coli communis* intravenously 0.25 c.c., May 10. Uranium nitrate subcutaneously 2 mg., May 13. *B. coli communis* intravenously 0.25 c.c., May 17. Uranium nitrate subcutaneously 2 mg., May 20. *B. coli communis* intravenously 0.25 c.c., May 23. Uranium nitrate subcutaneously 2 mg., May 26. *B. coli communis* intravenously 0.25 c.c., May 29. Uranium nitrate subcutaneously 2 mg., June 1. *B. coli communis* intravenously 0.25 c.c., June 4. Uranium nitrate subcutaneously 2 mg., June 7. *B. coli communis* intravenously 0.25 c.c., June 10. Uranium nitrate subcutaneously 2 mg., June 13. *B. coli communis* intravenously 0.25 c.c., June 16, etc. *B. coli communis* intravenously 0.25 c.c., June 26.

Killed July 8, 1912. Duration of experiment 106 days. Kidney shows slight focal increase in connective tissue, cellular in type.

RABBIT 442.—Weight, 1,560 grams. Caged March 9, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., March 10, 13, 16. *B. coli communis* intravenously 0.25 c.c. of a 24-hour bouillon culture, March 18, 21, 24. Uranium nitrate subcutaneously 2 mg., March 27, 30, April 2. *B. coli communis* intravenously 0.25 c.c., April 5, 8, 11. Uranium nitrate subcutaneously 2 mg., April 14, 17, 21. *B. coli communis* intravenously 0.25 c.c., April 24, 27, 30. Uranium nitrate subcutaneously 2 mg., May 4, 7, 10. *B. coli communis* intravenously 0.25



c.c., May 13, 17, 20. Uranium nitrate subcutaneously 2 mg., May 23, 26, 29. *B. coli communis* intravenously June 1, 4, 7.

Killed June 10, 1912. Duration of experiment 93 days. Kidney shows scattered areas of connective tissue increase with distinct dilatation of some of tubules.

RABBIT 445.—Weight, 1,530 grams. Caged March 10, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., March 11, 14, 16. *B. coli communis* intravenously 0.25 c.c. of a 24-hour bouillon culture, March 18, 21, 24. Uranium nitrate subcutaneously 2 mg., March 27, 30, April 2. *B. coli communis* intravenously 0.25 c.c., April 5, 8, 11. Uranium nitrate subcutaneously 2 mg., April 14, 17, 21. *B. coli communis* intravenously 0.25 c.c., April 24, 27, 30. Uranium nitrate subcutaneously 2 mg., May 4, 7, 10. *B. coli communis* intravenously 0.25 c.c., May 13, 17, 20. Uranium nitrate subcutaneously 2 mg., May 23, 26, 29. *B. coli communis* intravenously 0.25 c.c., June 1, 4, 7.

Killed June 10, 1912. Duration of experiment 92 days. Kidney shows quite large areas in which there is a very considerable connective tissue increase.

RABBIT 460.—Weight, 2,380 grams. Caged March 27, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., March 27. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, March 30. Uranium nitrate subcutaneously 2 mg., April 2. *B. coli communis* intravenously 0.25 c.c., April 5. Uranium nitrate subcutaneously 2 mg., April 8. *B. coli communis* intravenously 0.25 c.c., April 11. Uranium nitrate subcutaneously 2 mg., April 14. *B. coli communis* intravenously 0.25 c.c., April 17. Uranium nitrate subcutaneously 2 mg., April 21. *B. coli communis* intravenously 0.25 c.c., April 24. Uranium nitrate subcutaneously 2 mg., April 27. *B. coli communis* intravenously 0.25 c.c., April 30. Uranium nitrate subcutaneously 2 mg., May 4. *B. coli communis* intravenously 0.25 c.c., May 7. Uranium nitrate subcutaneously 2 mg., May 10. *B. coli communis* intravenously 0.25 c.c., May 13. Uranium nitrate subcutaneously 2 mg., May 17. *B. coli communis* intravenously 0.25 c.c., May 20. Uranium nitrate subcutaneously 2 mg., May 23. *B. coli communis* intravenously 0.25 c.c., May 26. Uranium nitrate subcutaneously 2 mg., May 29. *B. coli communis* intravenously 0.25 c.c., June 1. Uranium nitrate subcutaneously 2 mg., June 16. *B. coli communis* intravenously 0.25 c.c., June 20. Uranium nitrate subcutaneously 2 mg., June 23. *B. coli communis* intravenously 0.25 c.c., June 26. Uranium nitrate subcutaneously 2 mg., June 29.

Killed July 8, 1912. Duration of experiment 103 days. Kidney shows picture quite similar to Rabbit 445.

RABBIT 461.—Weight, 2,100 grams. Caged March 27, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., March 27. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, March 30. Uranium nitrate subcutaneously 2 mg., April 2. *B. coli communis* intravenously 0.25 c.c., April 5. Uranium nitrate subcutaneously 2 mg., April 8. *B. coli communis* intravenously 0.25 c.c., April 11. Uranium nitrate subcutaneously 2 mg., April 14. *B. coli communis* intravenously 0.25 c.c., April 18. Uranium nitrate subcutaneously 2 mg., April 21. *B. coli communis* intravenously 0.25 c.c., April 24. Uranium nitrate subcutaneously 2 mg., April 27. *B. coli communis* intravenously 0.25 c.c., April 30. Uranium nitrate subcutaneously 2 mg., May 4. *B. coli communis* intravenously 0.25 c.c., May 7. Uranium nitrate subcutaneously 2 mg., May 10. *B. coli communis* intravenously 0.25 c.c., May 13. Uranium nitrate subcutaneously 2 mg., May 17. *B. coli communis* intravenously 0.25 c.c., May 20. Uranium nitrate subcutaneously 2 mg., May 23. *B. coli communis* intravenously 0.25 c.c., May 26. Uranium nitrate subcutaneously 2 mg., May 29. *B. coli communis* intravenously 0.25 c.c., June 1. Uranium nitrate subcutaneously 2 mg., June 4. *B. coli communis* intravenously 0.25 c.c., June 7. Uranium nitrate subcutaneously 2 mg., June 10. *B. coli communis* intravenously 0.25 c.c., June 13. Uranium nitrate subcutaneously 2 mg., June 16. *B. coli communis* intravenously 0.25 c.c., June 20. Uranium nitrate subcutaneously 2 mg., June 23. *B. coli*

*communis* intravenously 0.25 c.c., June 26. Uranium nitrate subcutaneously 2 mg., June 29.

Killed July 8, 1912. Duration of experiment ninety-eight days. Kidney shows areas radiating in from surface in which there is considerable connective tissue increase, cellular in character with almost complete disappearance of tubules.

RABBIT 468.—Weight, 1,890 grams. Caged April 10, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., April 11. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, April 14. Uranium nitrate subcutaneously 2 mg., April 18. *B. coli communis* intravenously 0.25 c.c., April 21. Uranium nitrate subcutaneously 2 mg., April 24. *B. coli communis* intravenously 0.25 c.c., April 27. Uranium nitrate subcutaneously 2 mg., April 30. *B. coli communis* intravenously 0.25 c.c., May 4. Uranium nitrate subcutaneously 2 mg., May 7. *B. coli communis* intravenously 0.25 c.c., May 10. Uranium nitrate subcutaneously 2 mg., May 13. *B. coli communis* intravenously 0.25 c.c., May 17. Uranium nitrate subcutaneously 2 mg., May 20. *B. coli communis* intravenously 0.25 c.c., May 23. Uranium nitrate subcutaneously 2 mg., May 26. *B. coli communis* intravenously 0.25 c.c., May 29. Uranium nitrate subcutaneously 2 mg., June 1. *B. coli communis* intravenously 0.25 c.c., June 4. Uranium nitrate subcutaneously 2 mg., June 7. *B. coli communis* intravenously 0.25 c.c., June 10. Uranium nitrate subcutaneously 2 mg., June 13. *B. coli communis* intravenously 0.25 c.c., June 16. Uranium nitrate subcutaneously 2 mg., June 20. *B. coli communis* intravenously 0.25 c.c., June 23. Uranium nitrate subcutaneously 2 mg., June 26. *B. coli communis* intravenously 0.25 c.c., June 29.

Killed July 8, 1912. Duration of experiment 89 days. Kidney shows moderate degree of connective tissue increase, more marked in mid-zonal region and fairly cellular.

RABBIT 470.—Weight, 1,820 grams. Caged April 10, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., April 11. *B. coli communis* intravenously 0.25 c.c., of 24-hour bouillon culture, April 14. Uranium nitrate subcutaneously 2 mg., April 17. *B. coli communis* intravenously 0.25 c.c., April 21. Uranium nitrate subcutaneously 2 mg., April 24. *B. coli communis* intravenously 0.25 c.c., April 27. Uranium nitrate subcutaneously 2 mg., April 30. *B. coli communis* intravenously 0.25 c.c., May 4. Uranium nitrate subcutaneously 2 mg., May 7, etc., to June 10.

Found dead June 10, 1912. Duration of experiment fifty-nine days. Kidney shows a moderate degree of connective tissue increase quite diffuse in its distribution.

RABBIT 471.—Weight, 1,912 grams. Caged April 10, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., April 11. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, April 14. Uranium nitrate subcutaneously 2 mg., April 17. *B. coli communis* intravenously 0.25 c.c., April 21. Uranium nitrate subcutaneously 2 mg., April 24. *B. coli communis* intravenously 0.25 c.c., April 27. Uranium nitrate subcutaneously 2 mg., April 30. *B. coli communis* intravenously 0.25 c.c., May 4. Uranium nitrate subcutaneously 2 mg., May 7. *B. coli communis* intravenously 0.25 c.c., May 10. Uranium nitrate subcutaneously 2 mg., May 13. *B. coli communis* intravenously 0.25 c.c., May 17. Uranium nitrate subcutaneously 2 mg., May 20. *B. coli communis* intravenously 0.25 c.c., May 23. Uranium nitrate subcutaneously 2 mg., May 26. *B. coli communis* intravenously 0.25 c.c., May 29. Uranium nitrate subcutaneously 2 mg., June 1. *B. coli communis* intravenously 0.25 c.c., June 4. Uranium nitrate subcutaneously 2 mg., June 7. *B. coli communis* intravenously 0.25 c.c., June 10. Uranium nitrate subcutaneously 2 mg., June 13. *B. coli communis* intravenously 0.25 c.c., June 16.

Found dead June 20, 1912. Duration of experiment sixty-nine days. Kidney shows condition quite similar to Rabbit 470, but more marked in degree.



RABBIT 472.—Weight, 1,450 grams. Caged April 7, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., April 11. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, April 14. Uranium nitrate subcutaneously 2 mg., April 18. *B. coli communis* intravenously 0.25 c.c., April 21. Uranium nitrate subcutaneously 2 mg., April 24. *B. coli communis* intravenously 0.25 c.c., April 27. Uranium nitrate subcutaneously 2 mg., April 20. *B. coli communis* intravenously 0.25 c.c., May 4. Uranium nitrate subcutaneously 2 mg., May 7. *B. coli communis* intravenously 0.25 c.c., May 10. Uranium nitrate subcutaneously 2 mg., May 13. *B. coli communis* intravenously 0.25 c.c., May 17. Uranium nitrate subcutaneously 2 mg., May 20. *B. coli communis* intravenously 0.25 c.c., May 23. Uranium nitrate subcutaneously 2 mg., May 26. *B. coli communis* intravenously 0.25 c.c., May 29. Uranium nitrate subcutaneously 2 mg., June 1. *B. coli communis* intravenously 0.25 c.c., June 4.

Found dead June 5, 1912. Duration of experiment fifty-four days. Kidney shows condition quite similar to Rabbit 471.

RABBIT 473.—Caged April 20, 1912. Urine negative. Uranium nitrate subcutaneously 2 mg., April 21. *B. coli communis* intravenously 0.25 c.c. of 24-hour bouillon culture, April 24. Uranium nitrate subcutaneously 2 mg., April 27. *B. coli communis* intravenously 0.25 c.c., April 30. Uranium nitrate subcutaneously 2 mg., May 4. *B. coli communis* intravenously 0.25 c.c., May 7. Uranium nitrate subcutaneously 2 mg., May 10. *B. coli communis* intravenously 0.25 c.c., May 13. Uranium nitrate subcutaneously 2 mg., May 17. *B. coli communis* intravenously 0.25 c.c., May 20. Uranium nitrate subcutaneously 2 mg., May 23. *B. coli communis* intravenously 0.25 c.c., May 26. Uranium nitrate subcutaneously 2 mg., May 29. *B. coli communis* intravenously 0.25 c.c., June 1. Uranium nitrate subcutaneously 2 mg., June 4. *B. coli communis* intravenously 0.25 c.c., June 7. Uranium nitrate subcutaneously 2 mg., June 10. *B. coli communis* intravenously 0.25 c.c., June 13. Uranium nitrate subcutaneously 2 mg., June 16. *B. coli communis* intravenously 0.25 c.c., June 20.

Found dead June 21, 1912. Duration of experiment sixty-three days. Kidney shows quite marked connective tissue increase, diffuse in character and in addition areas of marked connective tissue increase radiating in from surface in which tubules have practically disappeared.

#### CONCLUSIONS

In rabbits that survive the immediate toxic effects of the agents employed, chronic renal lesions can be produced with considerable regularity by injections of a chemical (uranium nitrate) and a bacteria (*B. coli communis*). The lesions so produced are closely similar to those involving the renal tissues in chronic interstitial nephritis in man.

Carney Hospital.

STUDY XVIII: ACUTE RENAL LESIONS PRODUCED BY  
URANIUM NITRATE IN THE DOG IN COMPARISON  
WITH THE RABBIT \*

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In 1908, Christian<sup>1</sup> described the occurrence of hyaline droplets in the walls of the capillaries of the glomerular tuft in rabbits that had recently received doses of uranium nitrate. In 1911, a further study of this lesion was published.<sup>2</sup> At Dr. Christian's suggestion, I undertook to see whether a similar lesion could be produced in dogs by injection of uranium nitrate.

Nine dogs of varying breeds and weights were tested by urinary examination to exclude a possible spontaneous nephritis. They then were given subcutaneous injections of uranium nitrate. The number of doses and their time interval differed considerably, varying from a single dose of 3.3 mg. per kilo of body weight to three doses of 12 mg. per kilo of body weight. Except in two instances, the doses were given on successive days, and the animal killed twenty-four hours after the last dose by primary chloroform anesthesia and deflation of the lungs by incision of the diaphragm.

A study of the renal tissues shows that the hyaline droplets are much more difficult of production in dogs than in rabbits, and when they do occur as a rule, they are not confined so definitely to the wall of the capillary in the glomerular tuft. In the rabbit two or three doses of uranium nitrate 3.3 mg. per kilo of body weight, or larger, quite regularly produced extensive renal lesions, among them hyaline droplets in many of the glomeruli. In the dog, even with large doses of uranium nitrate, this glomerular lesion was infrequent, and with smaller doses such as produced in the rabbits the hyaline droplets with great regularity and in typical form, in the dog they did not occur. The epithelium of the tubules in the dog's kidney shows the same lesion as that found in the rabbit, degeneration or necrosis, with disintegration of the renal epith-

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\* A series of studies on experimental cardiovascular disease has been published previously; see Study XVI, *THE ARCHIVES OF INT. MED.*, 1913, xi, 517, for reference to these.

\* From the Laboratory of the Department of the Theory and Practice of Physic, Medical School, Harvard University.

\* This work was done under a grant from the Proctor Fund for the Study of Chronic Diseases.

1. Christian, H. A.: *A Glomerular Lesion of Experimental Nephritis*. Boston Med. and Surg. Jour., 1908, clix.

2. Christian, H. A.: *THE ARCHIVES OF INT. MED.*, 1911, viii, 469.

elium; but here again larger doses per kilo of body weight were required in the dog to produce the lesion that quite regularly results from smaller doses in the rabbit. A dose of uranium nitrate in a dog three and a half times as large as the one commonly employed in the rabbit seemed to produce no more extensive lesion. This indicates that the dog's kidney is distinctly more resistant to the acute action of uranium nitrate than is the rabbit's kidney. The results of the various experiments are given in the following protocols:

#### PROTOCOLS

ANIMAL 489.—Female spaniel dog. Weight, 8.3 K. Caged June 13, 1912. Urine negative. June 16 received subcutaneously 3.3 mg. uranium nitrate per kilo of body weight. Killed June 17.

Kidney showed here and there deep in the medulla a single tubule or group of straight or convoluted tubules, with moderate necrosis and beginning cast formation. The glomeruli showed no lesion.

ANIMAL 484.—Mongrel dog. Weight, 12 K. Caged May 27, 1912. Urine negative. May 28 and 29 received subcutaneously uranium nitrate 3.3 mg. per K. of body weight. Killed May 30.

Kidney showed very slight fatty and hyaline degeneration of the convoluted tubules, with more extensive necrosis of groups of convoluted and straight tubules than in Animal 489. Glomeruli showed no lesion.

ANIMAL 450.—Brindle mongrel dog. Weight, 8.7 K. Caged March 10, 1912. Urine negative. March 13, 14, 15, received subcutaneously 3.3 mg. uranium nitrate per K. of body weight. Killed March 15.

Kidney showed moderate degree of necrosis and hyaline degeneration of the convoluted tubules, with more extensive necrosis of the collecting tubules. Many casts. A few glomeruli showed very small hyaline droplets in the glomerular tufts, for the most part in the wall of the capillary.

ANIMAL 474.—Mongrel dog. Weight, 8.3 K. Caged May 8, 1912. Urine negative. May 12, 13, 14, received subcutaneously 5 mg. uranium nitrate per K. of body weight. Killed May 15.

Kidney showed degenerative and necrotic lesions more marked in degree than in Animal 450, but the glomeruli appeared normal.

ANIMAL 281.—Mongrel dog. Weight, 9 K. October 16 received subcutaneously 7 mg. uranium nitrate per K. body weight. Killed October 23.

Kidney showed marked necrosis of the convoluted tubules and straight tubules throughout the cortex. Many casts. Many of the glomeruli showed fine hyaline droplets, for the most part, but not entirely, in the walls of the capillaries.

ANIMAL 332.—Coach dog. Weight, 12.7 K. Caged Oct. 2, 1911. Urine negative. October 5 received subcutaneously 5 mg. uranium nitrate per K. body weight. This produced albuminuria and casts, reaching the maximum October 10, gradually decreasing and disappearing. November 4, weight 11 K. November 4, 5, 6, received subcutaneously 7 mg. uranium nitrate per K. body weight. Killed November 6.

Kidney showed moderate necrosis of the epithelium of the tubules. Glomeruli showed no lesion.

ANIMAL 374.—Collie dog. Weight, 14.5 K. Caged Jan. 2, 1912. Urine negative. January 23, 24, 25, 26, received subcutaneously 7 mg. uranium nitrate per K. body weight. Killed January 27.

Kidney showed marked necrosis of straight and convoluted tubules, not quite so marked as in Animal 375. Several glomeruli showed hyalin droplets, but these were not confined to the wall of the capillary.

ANIMAL 375.—Bull dog. Weight, 16 K. Caged Jan. 23, 1912. Urine negative. January 24, 25, 26, received subcutaneously 8 mg. uranium nitrate per K. of body weight. Killed January 27.

Kidney showed marked necrosis of tubular epithelium. Glomeruli showed many hyalin droplets, but few of these were definitely confined to the wall of the capillary.

ANIMAL 449.—Black and white mongrel female dog. Weight, 15.4 K. Caged March 10, 1912. Urine negative. March 13, 14, 15, received subcutaneously 12 mg. uranium nitrate per K. body weight. Killed March 16.

Kidney showed marked necrosis of tubular epithelium, though probably no more extensive lesion than shown by Animals 374 and 375. In the glomeruli hyalin droplets were more common than in the preceding animals, and occurred both in and outside of the walls of the capillaries.

In all of these animals uranium nitrate produced in the urine the signs of acute nephritis.

#### CONCLUSIONS

The dog's kidney seems distinctly more resistant to the action of uranium nitrate than the rabbit's in so far as the production of anatomical changes is concerned. The glomerular lesion with the formation of hyaline droplets in the wall of the capillary tuft is produced with great difficulty in the dog.

Carney Hospital.



# PARATYPHOID FEVER: A SEROLOGIC STUDY IN RELATION TO THE EPIDEMIOLOGY\*

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During the course of epidemiologic investigations of typhoid fever the importance of the variability of clinical manifestations, the frequent failure of physicians to establish a diagnosis satisfactory to themselves, and the large percentage of cases in which positive agglutination reactions with the *Bacillus typhosus* were absent, particularly in water-borne epidemics, led to the observations herewith presented. It is the purpose of this paper to offer as a contribution the facts developed by serologic studies in certain epidemics to which I was assigned in charge under the direction of Dr. Samuel G. Dixon, Pennsylvania State Commissioner of Health.

## GENERAL CONSIDERATIONS

By means of careful observations of the biochemistry and the agglutination properties of micro-organisms of the typho-colon type, it has been found that certain forms have characteristics which group them together; further, that their pathogenicity bears a somewhat definite relation to the grouping. For the purpose of study and in the light of present information, the classification based on the suggestion of Park<sup>1</sup> is used; in the order of pathogenicity it is as follows:

1. Typhoid group.
2. Paratyphoid group.
3. Dysentery group.
4. Colon group.

It is apparent from the literature that has accumulated since the first description of infections which are now known as paratyphoid fever that this disease is an unquestioned etiological entity. The bacilli—*B. paratyphosus A* and *B. paratyphosus B*—described as causing this pathological process have been divided into two types because of differences in cultural and agglutination phenomena. In the group to which they have been assigned, there are numerous other forms, the more important of which are *B. paracoli*, *B. alcaligenes*, *B. of hog cholera*, *B. suisepcticus*, *B. enteritidis* (Gaertner), *B. of mouse typhoid*, *B. of calves' diarrhea* and *B. psittacosis*. To this date there is evidence that only certain members

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\* From the Pennsylvania State Department of Health.

\* Submitted for publication March 3, 1913.

1. Park: Pathogenic Bacteria, 1910.

are pathogenic to man, and it is with these that we are particularly interested.

The clinical course of unreported cases has been considered indistinguishable from that of typhoid fever. It is not possible to present the results of clinical study in this contribution. There are many other features which remain to be clearly defined, such as the sources of infection, the methods of transmission, the association of the micro-organisms with the *B. typhosus* and the relation of clinical types to atypical forms of typhoid fever; it is desirable to know in what proportional relationship typhoid fever and paratyphoid fever and infections by other members of the typho-colon group may occur. It is also necessary for the application of preventive and therapeutic measures to know of what value anti-typhoid vaccine may be in cases of so-called mixed infections of this class, and, as to whether or not it would be advisable to use a polyvalent vaccine for the prevention of this group infection. These problems have, in part, been studied, and certain features will be considered in this article; others we hope to make the basis of future contributions.

The justification for separating paratyphoid fever from typhoid fever has been much discussed. In many epidemics of typhoid fever, particularly those in which the infection is water-borne, the earlier cases are atypical in onset and clinical course, and it is often difficult to convince the general practitioner that he is dealing with a disease which is bacillary in origin and which needs the same supervision as a typical case of typhoid fever.

My experience indicates that relatively few cases are diagnosed as typhoid fever. There is no doubt that many diagnoses of "catarrhal fever," "gastric fever," "grip," gastro-enteritis, abdominal influenza and others are made for patients suffering with a true *B. typhosus* infection, but far more frequently with infections by some pathogenic member of the paratyphoid group.

Each unreported case, escaping sanitary control, is the potential source of an epidemic and each case incorrectly diagnosed is inimical to the health of those acting as nurse and often as the household cook. In only one territory, that of Hawaii,<sup>2</sup> and in only one state, namely, Washington,<sup>3</sup> is it specifically required that the disease, as a clinical entity, shall be reported to the health authorities. In Pennsylvania, under the power granted to the Commissioner of Health, the latter may, with approval of the Advisory Board, declare a disease reportable. Such action has been taken during three epidemics which were under my immediate charge.

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2. U. S. Pub. Health Bull., No. 45.

3. Rules and Regulations Washington State Board of Health, 1912.

In speaking of early cases, it is not meant that the *incubation* period of paratyphoid fever is not as long as that of typhoid fever; we have some evidence that the period for true infections by *B. paratyphosus A* and *B. paratyphosus B* is the same as of *B. typhosus*. Rolly,<sup>4</sup> in his studies of the so-called gastric form of paratyphoid fever, states that one of the peculiarities of paratyphoid fever is the insignificant incubation time. However, his observations were made on some 250 individuals whose infection was said to be due to *B. typhosus B*, and, as will be noted later, there is some probability that this was really a *B. enteritidis* infection, in which the incubation period is doubtless shorter than in true paratyphoid infections. There is, however, a shorter *prodromal* period for paratyphoid infections; the onset is more abrupt and the clinical expression of invasion and multiplication is more varied. The differentiation should be sharply made between the incubation period and the prodromal period.

Variations in clinical form lead to resemblances to many other types of infection. It is not desirable to discuss such differences in this paper, but in order to emphasize the need for a careful clinical and bacteriological diagnosis, the disease, *paratyphoid fever*, may be said to present one of four general types:

1. That closely resembling typhoid fever; this is most often due to *B. paratyphosus A*.
2. That closely resembling influenza of the abdominal type. This may be due to *B. paratyphosus A* or *B* or to mixed infections including these and *B. typhosus*, as well as *B. para coli* or *B. alcaligenes*.
3. That suggesting a general gastro-enteric inflammation with nausea and vomiting. This is often diagnosed as ptomain poisoning and is due usually to one of the lower group members of the paratyphoid group.
4. That closely resembling dysentery, in which, however, *B. dysenteriae* is not found but most often *B. enteritidis* (Gaertner) is recovered from the feces and the agglutination reaction confirms the findings. According to the literature *B. paratyphosus B* is almost as frequently the offending micro-organism. This type, too, is often diagnosed as ptomain poisoning and almost as frequently "cholera morbus."

#### SOURCE

In considering the source of infection it is often difficult to limit the discussion to the true paratyphoid forms. There is no doubt from many observations which have been recorded that the various members of the paratyphoid group come from the intestinal tracts of domestic animals.

*B. paratyphosus A*.—This micro-organism in water-supplies was first isolated and identified from the spring water of an Italian village by Paladino-Blandino<sup>5</sup> in 1903. However, Schottmüller<sup>6</sup> ascribes six cases

4. Rolly, Fr.: München. med. Wehnschr., 1911, p. 559.

5. Paladino-Blandini: Ann. d'ig. sper., 1903, xv, 159.

6. Schottmüller: Deutsch. med. Wehnschr., 1900, p. 511; Ztschr. f. Hyg. u. Infektionskrankh., 1901, Bd. xxxvi; München. med. Wehnschr., 1902, No. 38.

reported by him up to 1901 to transmission by water. May,<sup>7</sup> in 1909, was successful in isolating the same form from running water which was used as the source of domestic supply. There are no other records of its recovery from drinking supplies.

Baermann and Eckersdorff,<sup>8</sup> while studying eight cases, isolated cultures from the feces. Harvey,<sup>9</sup> working alone, recovered the organism from the blood of eleven patients and many times from the feces, and later, working with Grattan<sup>10</sup> in the study of two small outbreaks, recovered the organism from the blood of seven patients and traced the source of infection to a cook who had suffered with cholecystitis several months previously, and whose blood showed a positive agglutination with the organism recovered from the other patients. Purjesz<sup>11</sup> isolated cultures from both feces and urine, and Aoki<sup>12</sup> recovered the organism in almost pure culture from an abdominal abscess and the feces. This patient was shown to be a carrier case and is so discussed by them. Monnier and Riberau<sup>13</sup> report an interesting recovery with autopsy, showing a positive agglutination for *B. paratyphosus A* in a dilution of 1 to 200, negative to *B. paratyphosus B*. The organism was recovered from the blood-stream, and on autopsy, was found in the spleen, liver and kidneys. However, isomutatoric tests were not made in this instance.

*B. Paratyphosus B*.—The identification of *B. paratyphosus B* does not seem, to the present time, to be distinctly certain, and, in the review of the literature as presented, note is not made of the failure to differentiate sharply between this organism and *B. enteritidis* (Gaertner). The recent studies by Bainbridge would indicate that the particular strain of *B. paratyphosus B* recovered from cases of meat poisoning is entirely different from the true *B. paratyphosus B* referred to in the studies of this article. Bainbridge believes that all cases of meat poisoning are really infections by *B. enteritidis* (Gaertner).

*B. paratyphosus B* was first described by Achard and Bensaude<sup>14</sup> in 1896, and has been frequently recovered from the blood and dejecta of those ill with the disease. The first epidemic reported in the literature occurred in Saarbrücken,<sup>6</sup> Germany, in 1902, during which the bacillus was recovered from the feces in eighteen cases, and from the blood in four cases. Since that date many studies on organisms recovered from

7. May: Jour. Roy. Inst. of Pub. Health, 1909, xvii, 551.

8. Baermann and Eckersdorff: Berl. klin. Wehnschr., 1909, No. 40.

9. Harvey: Jour. Roy. Army Med. Corps, 1910, xiv, No. 4.

10. Grattan and Harvey: Jour. Roy. Army Med. Corps, 1911, xvi, No. 1.

11. Purjesz: Wien. klin. Wehnschr., 1910, No. 36, p. 1284.

12. Aoki: Centrabl. f. Bakteriöl., 1 Orig. 1910, lvi, 110.

13. Monnier and Riberau: Compt. rend. Soc. de biol., 1910, lxi, 151.

14. Achard and Bensaude: Bull. et mém. Soc. méd. d. hôp. de Paris, 1896, xiii, 820.



water-supplies have been made. Gaechtgens<sup>15</sup> reports its isolation on three different occasions from a public water-supply, the source of which was a stream supplying an Alsatian municipality. In another instance, he recovered both *B. paratyphosus B* and *B. alcaligenes* from a fountain-supply.

Epidemics from ingestion of infected ice cream have been reported. Prigge and Sachs-Muke<sup>16</sup> studied thirteen cases in one outbreak. Liebetrau<sup>17</sup> reported an outbreak of nineteen cases of which three patients died; a nurse living in the home of a confectioner had nursed a paratyphoid patient. Both the nurse and the confectioner's helper showed a positive agglutination with *B. paratyphosus B*, and the epidemic followed the ingestion of ice cream manufactured by the helper.

Popps<sup>18</sup> reports paratyphosus B fever following the ingestion of spoiled oysters, the diagnosis being based on positive agglutination with *B. typhosus* and *B. paratyphosus B*; however, no other studies were made and the organism was not recovered from the blood or feces. König<sup>19</sup> studied four patients who, while at a convention, ate raw ham and whose blood gave a positive reaction with *B. paratyphosus B*. He later isolated the same organism from the ham. Friedrichs and Gardiewski<sup>20</sup> recovered a paratyphoid-like organism from tripe. It was not definitely identified. Rommeler<sup>21</sup> recovered the organism from pudding, sausage and tripe. Prigge and Sachs-Muke,<sup>16</sup> in another instance, studied sixteen cases of paratyphoid fever, apparently infected by roast swine. However, there were no bacteriological studies. Zimmerman<sup>22</sup> recovered *B. paratyphosus B* from one patient infected from eating the meat of a previously sick goose, and also an epidemic of sixteen cases from eating the meat of three calves which had previously suffered from calves' diarrhea. Heinemann<sup>23</sup> and Neumann<sup>24</sup> studied forty-seven cases in Cassel and found that only those who had eaten meat from a certain butcher shop gave a positive agglutination reaction with *B. paratyphosus B*. Rolly<sup>4</sup> duplicated this experience in a study of 250 patients, all of whom suffered from a gastric form. Neumann<sup>24</sup> reports its recovery from the blood, and Jensen and Kock<sup>25</sup> isolated a typical form from the bone sinus of an old abscess.

15. Gaechtgens: Arb. a. d. k. Gsndhtsamte, 1909, xxx, Part 3, p. 610.

16. Prigge and Sachs-Muke: Klin. Jahrb., 1909, xxi.

17. Liebetrau: Ztschr. f. med. Beamte., 1910, No. 2.

18. Popps, Fritz: München. med. Wchnschr., 1910, p. 584.

19. König: Centralbl. f. Bakteriöl., Part 1, Orgl., 1909, 1.

20. Friedrichs and Gardiewski: Centralbl. f. Bakteriöl., Part 1, Orgl., 1909, li, 509.

21. Rommeler: Centralbl. f. Bakteriöl., Part 1, Orgl., 1909, li, 501.

22. Zimmerman: Ztschr. f. med. Beamte., 1910, No. 3.

23. Heinemann: Ztschr. f. med. Beamte., 1911, Part 1, p. 2.

24. Neumann: Ztschr. f. med. Beamte., 1911, Part 1, p. 2.

25. Jensen and Kock: Deutsch. med. Wchnschr., 1910, p. 2196.

The evidence that the organism exists in the intestinal tracts of domestic animals has been demonstrated in at least three instances. Schmidt<sup>26</sup> and Conradi<sup>27</sup> recovered the organism from the feces of swine, and in 1911, Ruediger<sup>28</sup> recovered the organism from the feces of a dog. A private communication has been recently received which reports the recovery of the organism from the feces of the domestic cat. This organism was properly identified by all of the accepted criteria.

While there is some confusion as to the proper identification of *B. paratyphosus* B, in those instances in which bacteriological identification may be acceptable, organisms unquestionably belonging to the paratyphoid group have been recovered from the meat of calves, geese, swine, from sausage, ham, tripe, mince meat, Hamburger steak, from spoiled oysters, ice cream, public water-supplies and also from the intestinal discharges of certain domestic animals; they have been recovered from blood, feces, urine, abdominal and periosteal abscesses, ovarian carcinoma,<sup>29</sup> in pyelonephritis associated with urinary calculi,<sup>30</sup> and on autopsy, from the liver, spleen and kidneys of human beings. With this extraordinary array of infectious foci it is not to be regarded as curious that the cause of atypical forms of typhoid fever should be regarded by many writers as ubiquitous.<sup>31</sup>

#### FREQUENCY

The relative frequency of paratyphoid infections has been studied without definite conclusions. Gwyn's<sup>32</sup> case was the only one of 265 which did not react positively with *B. typhosus*. Schottmüller<sup>6</sup> and Kurth,<sup>33</sup> in their series of cases, found twelve which were negative to *B. typhosus*, but reacted positively to *B. paratyphosus*. Johnston<sup>34</sup> studied four cases occurring during an epidemic of 194 cases of typhoid fever. Hewlett<sup>35</sup> found one in twenty-six cases. Hühnermann,<sup>36</sup> at Saarbrücken studied thirty-eight cases of paratyphoid infection and found no cases of typhoid infection. Conradi<sup>37</sup> established a diagnosis of paratyphoid fever in twenty-nine of 250 cases, otherwise diagnosed as typhoid fever.

26. Schmidt: München. med. Wehnschr., 1911, p. 563.

27. Conradi: Klin. Jahrb., 1909., xxi, Part 2.

28. Ruediger: Bull. Manila Med. Soc., June, 1911.

29. Seiffert: Med. klin. Berlin., March, 1912, viii, No. 9.

30. Spassokukozky: Wein. klin. Wehnschr., March, xxv, No. 13.

31. Hubener: Deutsch. med. Wehnschr., 1910, p. 70.

32. Gwyn: Bull. Johns Hopkins Hospital, 1898, p. 54.

33. Kurth: Deutsch. med. Wehnschr., 1901, p. 501.

34. Johnston: Am. Jour. Med. Sc., August, 1902.

35. Hewlett: Am. Jour. Med. Sc., August, 1902.

36. Hühnermann: Ztschr. f. Hyg. u. Infektionskrankh., 1902, p. 522.

37. Conradi: Deutsch. med. Wehnschr., xxx, No. 32; Ztschr. f. Hyg. u. Infektionskrankh., 1903, p. 141.

In 1905, Fox,<sup>38</sup> in his review of the literature, found that the reported cases at that time were approaching a hundred, the diagnosis of some being complete, while that of others was questionable. They were, in part sporadic, in part small epidemics, and he measures the frequency of occurrence by the cases of apparent typhoid fever presenting a negative Gruber-Widal reaction. These, according to the Johns Hopkins Hospital report, amounted to 2 per cent. of all cases, while Schottmüller's<sup>6</sup> first study of sixty-nine cases of apparent typhoid fever showed that 4 per cent. were paratyphoid infections. All cases studied were sporadic and were those regularly admitted to the hospital from various localities in which, so far as is stated, the disease was not epidemic.

Proescher and Roddy,<sup>39</sup> in 1910, in a review of the literature up to that date, attempted to separate reported known cases of paratyphoid A from paratyphoid B, and reported that in the Allegheny County General Hospital, during 1908-09 "over fifty cases of paratyphoid A, about 200 cases of typhoid, but not one cases of paratyphoid B, were diagnosed." According to this report, 20 per cent. of all apparent typhoids were paratyphoid A infections.

Criteria for the proper identification of *B. paratyphosus A* and *B* were followed in but few of the cases studied early (prior to the work of Fox). However, the measure of incidence of either *paratyphosus A* or *paratyphosus B* infections has not yet been determined, and its relative frequency will differ for every outbreak in which there is a mixed infection.

#### HISTORICAL

ISELIN.—While studying an outbreak of typhoid fever in Iselin, Indiana County, during February, 1911, the atypical clinical course of cases which had been tentatively diagnosed by local physicians as typhoid fever and Asiatic cholera led to a somewhat restricted study of the blood of nineteen patients.

At the time it was impossible to secure blood-cultures and the agglutinating properties of the serum of the nineteen patients were studied with four members of the typho-colon group, viz., *B. typhosus B. paratyphosus*, *B. paracoli* and *B. enteritidis* (Gaertner). Seven agglutinated the *B. typhosus* and twelve the *B. paratyphosus B*, and were negative to all other members of the group studied. The reactions were studied in but one dilution and time period, namely, 1-50 for one hour.

Through a misunderstanding, the feces were examined only for the cholera vibrio,<sup>40</sup> and for that reason no micro-organisms of the typho-colon group were recovered.

38. Fox: Univ. of Penn. Med. Bull., April, 1905.

39. Proescher and Roddy: THE ARCHIVES INT. MED., 1910, v, 263.

40. Hetsch: Klin. Jahrb., 1907, xvi, p. 187, has observed that cases of paratyphoid infection, accompanied with severe diarrhea, may be mistaken for Asiatic cholera.

The twelve reacting with *B. paratyphosus B* were, all but one, of the type diagnosed as "Asiatic cholera"; that one patient, instead of having diarrhea, showed marked constipation, subnormal temperature, prostration and death.

The mortality in this outbreak, in which there were clinically diagnosed twenty-two cases of typhoid fever and thirty-one of paratyphoid fever, showed that four, or 12.5 per cent., of the latter resulted fatally; while in ten, or 45 per cent. of the cases diagnosed as typhoid fever the patients did not recover. However, it should be pointed out that in the majority of cases in which death occurred and where typhoid fever had been diagnosed clinically, the patients had been referred to a hospital where no serologic studies were made, and there is a possibility of error in these figures. The four cases of paratyphoid fever which resulted fatally, had all been diagnosed serologically, as well as clinically.

BETHLEHEM.—During the subsidence of an epidemic of bacillary dysentery in Bethlehem during September, 1911, a series of eighteen cases were studied. The agglutination reaction of six of these was positive with *B. typhosus* only; one was positive with both *B. typhosus* and *B. paratyphosus B*, while eleven agglutinated *B. paratyphosus B* only. Negative reactions were obtained with *B. paratyphosus A*, *B. paracoli*, *B. enteritidis* (Gaertner), *B. suicida* and *B. alcaligenes*.

From the feces, in addition to the common forms, there were recovered *B. suicida*, *B. enteritidis* (Gaertner), *B. acidi lactici*, and two strains described by Kruse as *B. paradoxus* and *B. pseudotyphoid*. From the spring source of the public water-supply, which was the transmission agent causing the epidemic, *B. alcaligenes*, *B. suicida*, *B. acidi lactici*, *B. coli communior* and a dysentery form (*B. Rosen*) were recovered and identified in the usual way.

Two of the patients showing positive reactions to paratyphoid organisms died, a mortality of 16.6 per cent. There were 102 cases of typhoid fever reported during the last four months of the year in which the eighteen cases studied were included. The latter were the first to develop after an epidemic of dysentery<sup>41</sup> had passed its acme, and all showed atypical onsets and clinical courses. Subsequent cases infected at the same time gave a history of a longer prodromal stage and were typical *B. typhosus* infections.

BLANDON.—In an outbreak of thirty-five cases in Blandon, Berks County, during November and December, 1911, six atypical cases were studied. The blood of one reacted with *B. paratyphosus A* only, one with *B. typhosus*, *B. para coli* and *B. paratyphosus B*, and four with *B. typhosus* only. Of five micro-organisms recovered from the feces of

41. Hunt: Jour. Am. Med. Assn., 1912, lix, 919.



five cases, two proved to be *B. typhosus*, one was identified as *B. paratyphosus A*, one was *B. paratyphosus B* and one *B. enteritidis* (Gaertner).

COATESVILLE.—One Feb. 11, 1912, I was assigned to visit Coatesville, Chester County, for the purpose of establishing a diagnosis. Ten cases were studied and a tentative diagnosis of typhoid fever was made in three cases, and of paratyphoid fever in seven cases.

An investigation of the conditions in Coatesville showed that in the practice of nine physicians there were 131 cases in which a diagnosis had not been definite, many of the physicians, however, suspecting that they were dealing with an atypical type of typhoid fever; none of the cases had been reported. There are eighteen physicians in Coatesville, the 131 cases occurring in the practice of 50 per cent.

Facts which were known to the state department of health, in connection with evidence collected on the same date showed that it was a water-borne infection, and the definite source of pollution was found to be from human sewage, some of it from patients clinically diagnosed as having typhoid fever; in addition the water-supplies were found to be grossly polluted with sewage from domestic animals. The history of a case of meat poisoning or of paratyphoid fever could not be obtained at the polluting sources. The gross pollution of the public water-supply occurred between Jan. 19 and Jan. 23, 1912. The earliest date of onset was January 19; 334 cases were reported as typhoid fever to the local board of health, the mortality of which was thirty-five, or 10.5 per cent.; of those diagnosed serologically, the mortality was three, or 0.9 per cent., of these one reacted positively with *B. typhosus* only, one with *B. paratyphosus A* only, and one with *B. typhosus* and *B. paratyphosus A*.

Studies of the water, feces and blood of seventy-six patients were immediately undertaken, because of the many atypical cases which occurred.

The water examination showed the presence of large numbers of *B. paradoxus* (Kruse), *B. coli communis* and *B. cloacae*. No other members of the typho-colon group were recovered. The three types recovered were taken from samples obtained from the settling basin, the borough water mains and Newlin's Run, which was the polluted source of the epidemic.

No members of the typho-colon group were recovered from the few specimens of feces studied.

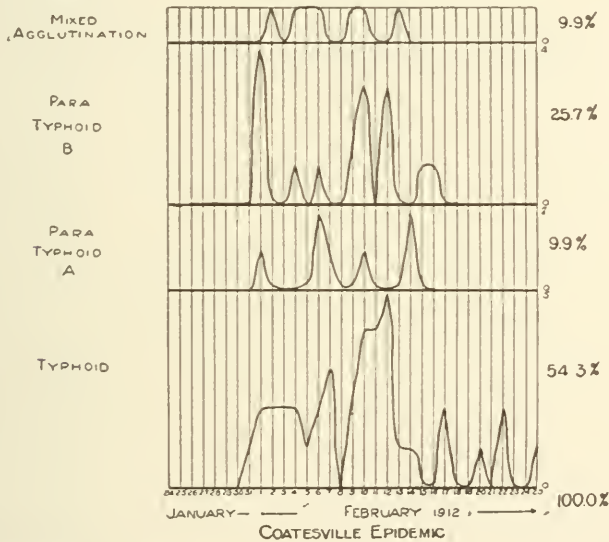
Blood-cultures were made, but with negative results. No case could be found which did not show a positive serum reaction. It is probable that the specific micro-organism had disappeared from the blood because of the presence of antibodies appearing coincident with the formation of agglutinins.

The seventy-six cases gave positive serum reactions in the following proportion:

- 54.5 per cent. were positive with *B. typhosus* only.
- 25.7 per cent. were positive with *B. paratyphosus B* only.
- 9.9 per cent. were positive with *B. paratyphosus A* only.
- 9.9 per cent. were positive with more than one group, members as follows:
- 1.4 per cent. *B. typhosus* and *B. paratyphosus A*.
- 5.7 per cent. *B. typhosus* and *B. paratyphosus B*.
- 1.4 per cent. *B. paratyphosus A* and *B. para-coli*.
- 1.4 per cent. *B. paratyphosus B* and *B. para-coli*.

It should be stated that these patients were selected at random (with reference to atypical characteristics) and were not chosen from any one group or type. They represent cases with dates of onset from January 19 to February 25, covering as wide a range as possible.

The dates of onset of the seventy-six cases are plotted in the accompanying diagram.



Agglutination reactions in the sera of seventy-six patients showing the relation of positive reactions to dates of onsets of the disease.

It will be observed that the curves representing the dates of onset of each group coincide to a very remarkable degree and would seem to indicate that the incubation period for the various members of the groups studied is practically the same. However, the prodromal stage, in all cases showing a reaction with more than one organism, and those giving a reaction with *B. paratyphosus B* only, was short and atypical; the onsets were abrupt.

The serums of all cases, in addition to the above, were studied with *B. enteritidis* (Gaertner), *B. suicida*, *B. dysenteriae* (Shiga) and *B. Rosen*. The results were negative in every instance except as given in the table.

In order that we might have an accurate idea as to the specificity of the reactions noted in this table, the agglutination reaction for each laboratory organism used was studied with serums from supposedly healthy persons. There was no agglutination of any of the types studied in dilutions of 1-10, 1-20, 1-40, 1-80 and 1-100, except that in one person a slight tendency to clumping without loss of motility of *B. typhosus* occurred in dilutions of 1-10. All control cultures were decidedly motile after one hour's observation.

The organisms used were obtained from the following sources: *B. typhosus* from the Philadelphia Bureau of Health Laboratory; *B. paratyphosus A* from the Laboratory of Hygiene, University of Pennsylvania; *B. paratyphosus B* from the same source, but was obtained by them from Dr. Cushing of Boston; *B. paracoli* from Dr. Buxton, *B. dysenteriae* (Shiga) was obtained from the Laboratory of Hygiene, University of Pennsylvania; *B. enteritidis* (Gaertner) from the same laboratory; *B. spicida* was isolated from feces in the Chester dysentery<sup>42</sup> outbreak in 1910 by Herbert Fox and J. B. Rucker, Jr., while *B. Rosen* was isolated from Bethlehem<sup>41</sup> water and feces during September, 1911, by Rucker.

#### SUMMARY

To recapitulate: During the course of four water-borne epidemics of typhoid fever, including 509 cases, 117, or 22.9 per cent., presented atypical features, either during the onset or clinical course.

From the feces of eleven patients, in addition to the common inhabitants of the gastro-intestinal tract, there were recovered *B. suicida*, *B. enteritidis* (Gaertner), three times; *B. acidi lactici*, twice; *B. paradoxus* (Kruse), twice; *B. pseudotyphosus* (Kruse), once; *B. typhosus*, twice; *B. paratyphosus A*, once; *B. paratyphosus B*, once, and *B. cloacae*, once.

From the water (in one epidemic) were recovered *B. alcaligenes*, *B. suicida*, *B. acidi lactici*, *B. coli communior* and a dysentery organism identified as one described by Rosen in an epidemic of bacillary dysentery at Rotterdam.

The agglutination reactions of the blood-serums from the 117 patients were studied in 1-50 dilutions and gave the following results during a period of one hour or less:

Micro-Organisms.	Number.	Per cent.
<i>B. typhosus</i> only.....	54	46.15
<i>B. paratyphosus A</i> only.....	9	7.7
<i>B. paratyphosus B</i> only.....	47	40.2
<i>B. typhosus A</i> and <i>B. paratyphosus</i> .....	1	0.86
<i>B. typhosus</i> and <i>B. paratyphosus B</i> .....	2	1.71
<i>B. paratyphosus A</i> and <i>B. paracoli</i> .....	2	1.71
<i>B. paratyphosus B</i> and <i>B. paracoli</i> .....	1	0.86
<i>B. typhosus B</i> , <i>B. paratyphosus B</i> and <i>B. paracoli</i> .....	1	0.86
	117	100.00

42. Report of Commissioner of Health, Pennsylvania, 1910.

In consideration of the foregoing, the following questions naturally suggest themselves:

1. What relation does a negative agglutination with *B. typhosus* bear to the presence of paratyphoid fever?
2. What is the influence of "group" agglutinins?
3. How nearly specific is the Gruber-Widal reaction as practiced in the usual hospital and private laboratory service?

The answer to the first question depends on a number of factors and includes a consideration of the second question. The proportion of negative reactions with *B. typhosus*, as has been previously noted, is very low (according to Fox,<sup>38</sup> 0.4 per cent. of proven cases); the basis for estimation, however, was from cases, all of which were collected from the literature, and included but one outbreak of any magnitude (Saarbrücken), the origin of which was found to be in meat. No water-borne epidemic of any size which included a study of atypical cases has been reported; however, as Jordan<sup>43</sup> suggests, both typhoid and paratyphoid fever may exist undifferentiated in the same epidemic, and the full extent of water infection for para cases is not fully recognized. The proportion of negative results in the Coatesville epidemic seems to confirm this suggestion, since 38.4 per cent. of cases were at all times negative with *B. typhosus*.

#### TIME OF APPEARANCE

Agglutinins in typhoid fever have been closely studied; they appear as early as the fifth day of disease, but may not appear until the fourth week, or even not until during the second month; various statements as to their time of appearance have been made by various observers. A compilation of these observations resulted as follows: In from 18 to 25 per cent. of cases they appeared during the first week (i. e., after the fifth day), 60 to 65 per cent. during the second week, 80 to 90 per cent. during the third, and upwards of 94 per cent. during the fourth and subsequent weeks. No observations are recorded as to the appearance of agglutinins peculiar to paratyphoid infections. However, there is no reason to believe that the same proportional relation to time of appearance would be different from that found in typhoid fever.

In the Coatesville epidemic, agglutinins reacting positively with the micro-organisms named in the following table were present on the days after onset as indicated:

<i>Paratyphosus A</i>	
Days after onset first observed.	Number of cases.
5 .....	1
9 .....	2
12 .....	1
13 .....	2
23 .....	4

43. Jordan: General Bacteriology, 1911.



<i>Paratyphosus B</i>	
Days after onset first observed.	Number of cases.
5 .....	1
8 .....	1
9 .....	2
10 .....	1
11 .....	1
13 .....	2
14 .....	3
15 .....	2
18 .....	4
21 .....	1
22 .....	2
39 .....	3
Unstated .....	1
<i>B. typhosus and B. paratyphosus A</i>	
5 .....	1
12 .....	1
<i>B. typhosus and B. paratyphosus B</i>	
17 .....	1
18 .....	2
19 .....	1
24 .....	4
<i>B. paratyphosus A and B. paracoli</i>	
13 .....	1
<i>B. paratyphosus B and B. paracoli</i>	
14 .....	1

It seems justifiable to assume that the time of appearance of agglutinins in paratyphoid does not differ from typhoid fever.

#### SPECIFICITY

It is possible that group agglutinins may account for some of the results obtained, but there are reasons for accepting the relative specificity of the reactions recorded. First, because the agglutination reaction of a laboratory strain of *B. typhosus* has been shown to be greater than for any related micro-organisms with which it has common or group agglutinins (such as *B. coli*, *B. enteritidis*, *B. paratyphosus B*); second, the power of a given serum to agglutinate *B. typhosus* in high dilutions practically always will be accompanied by the power to agglutinate the related bacilli in a much lower dilution; that is, the specific agglutinins in *B. typhosus* are usually far in excess of the non-specific or group agglutinins; and, third, group agglutinins are less likely to be present in normal serums. All studies reported in the literature have been made with immune serums.<sup>1, 4, 38, 43, 44, 45</sup> The variation of specific and group agglutinins has been noted, not only in bacteria biologically related, but

44. Hiss and Zinsser: Bacteriology, 1910.

45. Rimpau: Munchen. med. Wehnschr., 1909, p. 1843.

46. Wolff: Centrabl. f. Bakterirol., 1899, xxv.

also in different strains of the same organism.<sup>1, 46</sup> This variation, however, in view of the fact that specific agglutinins are always far in excess of group agglutinins should not detract from the specificity of these observations.

Jordan<sup>43</sup> points out the fact that the specific agglutinins produced by the two types of para-organisms, as those of *B. typhosus*, are entirely different. While Park<sup>1</sup> states that because of the lack of absolute specificity, the diagnosis of the type of infection or the absolute identification of the micro-organism can only be determined (through agglutination or bacteriolytic tests) with a certain degree of accuracy.

Various criteria for determining the specificity of the infecting micro-organism have been suggested.

Fox<sup>38</sup> states that "the best proof of the existence of a para-infection is the isolation of the bacterium, the saturation test, and, last, the bactericidal action."

Mayer<sup>43</sup> suggests the use of the Pfeiffer test (bactericidal) when there is a negative agglutination and atypical clinical course with recovery of *B. paratyphosus B* from the feces. Libram<sup>47</sup> emphasizes the value of the saturation test to differentiate infections by *B. typhosus* and *B. Gaertner*. Neuman<sup>24</sup> relied in the diagnosis of sixty cases on the recovery of a micro-organism from the blood and positive agglutination. Kallmeyer<sup>48</sup> reports one case in which he obtained a positive agglutination with *B. paratyphosus A* in 1-40 and 1-60 dilutions, and negative reaction with *B. typhosus*.

Grimm<sup>49</sup> states that the Gruber-Widal reaction in its mutual relations is specific in the early stage, but believes that a single examination often fails to fix the diagnosis between typhoid and paratyphoid infections because of atypical reactions caused by preformed agglutinins. He states that the occasional determination in high dilution in normal human serums for paratyphoid fever shows just as little as the recovery of the bacillus from the feces.

Hiss<sup>44</sup> decides that the specificity of reactions for practical purposes is not destroyed if proper dilution is carried out, as the degree of agglutinin formation is always higher for the specific bacterium than for allied micro-organisms. His diagnostic criteria would then depend on recovery of the bacillus and the study of immune serums.

Park<sup>1</sup> states that "the only reliable criteria for diagnosis" are negative reaction with *B. typhosus* in a dilution of not less than 1-40 with a positive reaction to a paratyphoid bacillus the agglutinability of which is known, or the recovery of a para form from the blood, urine or complicating inflammatory process.

47. Libram: Ztschr. f. Hyg. u. Infektionskrankh., 1909, lxiv, Part 3, p. 411.

48. Kallmeyer: St. Petersburg med. Wehnschr., 1909, No. 25.

49. Grimm: Centrabl. f. Bakteriolog., Abt. 1, Orgl., 1910, liv, Part 4, p. 367.

From these varied opinions it is difficult to conclude what criteria are acceptable. As Jordan points out, the absorption of specific agglutinins with the homologous organism removes not only the specific, but also part or all of the group agglutinins, and that bacteria may even remove agglutinins which they have not produced and which do not visibly affect them. Hence, the use of Castellani's test may lead to wrong conclusions unless high enough dilutions can be used to exclude all action of group agglutinins. The use of this test to determine "mixed infections" may, for the same reasons, lead to false deductions.

There is reason to believe that the bactericidal power of serums bears no relation to the presence of agglutinins. This has been apparently carefully worked out by the use of Pfeiffer's test and by studies on the relationship between bacteriolysins and agglutinins.

The recovery and absolute identification of the causative micro-organism is of unquestionable value, especially when isolated from the blood-stream. That this can be done without elaborate technic is shown in the report of Schweinburg,<sup>50</sup> who, while examining 129 specimens of blood collected for the agglutination reaction in the usual way, made cultures with the following results:

Week of Disease	No. of Cases	Positive Culture	Per Cent.	Positive Agglutination	Per Cent.
1	67	65	97	57	84.9
2	40	32	80	36	90
3	10	5	50	8	80
After third	12	Neg.	..	Neg.	..

In four specimens the organism isolated was identified as *B. paratyphosus A*; in all others, *B. typhosus*. (The tabulation is mine.)

The recovery of *B. paratyphosus A* or *B* from the dejecta seems less conclusive<sup>10</sup> since a variety of non-pathogenic types are also recovered from the feces of a relatively large number of domestic animals and humans. Given the presence of distinct clinical phenomena and the appearance of agglutinins in the blood-serums, the recovery of an organism from the feces has the greater value.

#### LABORATORY USAGE

The answer to the third question is found in the consideration of the Coatesville tabulations. Practically but few laboratories do much more than study submitted specimens of blood-serums with *B. typhosus*, usually in low dilutions — 1-20 or 1-40. As a rule, however, the agglutination reaction of a laboratory strain of known agglutinability (as noted above) is greater than for related micro-organisms, and there is no opportunity for interagglutination of a lower group member confusing a positive

50. Schweinburg: Wein. klin. Wchnschr., 1910, No. 9.

diagnosis. It, however, makes no provision for a negative result in the possible instance of lower group infection. We are justified in assuming that the reactions as routinely practiced in hospital and other laboratories are only as specific as the presence of typhoid fever is in the cases examined, and hence there is additional reason for determining the reaction in negative cases with members of the paratyphoid group. During the past two years this work has been carried out in the laboratories of the Pennsylvania State Department of Health, but only in epidemic work.

#### CONCLUSIONS

These studies during the course of four epidemics indicate that we were dealing with unusual and widely varied types. It is probable that each type was specific, if Park's criteria are acceptable. The serums, collected at varying periods of from seven to thirty-seven days, studied in dilutions of 1-50 with known organisms for at least one hour, with as many as three studies in several cases, reacted only with the types recorded; hence the specificity of the infecting bacilli as determined by these tests is probably accurate.

Many observers express the opinion that the differentiation of paratyphoid infections from typhoid fever is of importance only from an etiological standpoint. It would appear that the differentiation has a much greater significance. The rôle in transmission of the undiagnosed cases (as in the bacillus carrier) has not been statistically determined. If in fifty-nine of 509 cases, or 11.5 per cent., of all patients, there is a constant negative Gruber-Widal, it is of greatest importance to show the relation of this to some other and just as serious infection.

The doubt of many practitioners of the existence of atypical cases is more prevalent than can be easily understood and is very apparent to the sanitarian, often to an uncomfortable extent, since he must secure results through the *modus operandi* of legal procedure, or, at least of persuasion, and the difficulties in diagnosis are reflected in the difficulties to secure proper reporting and supervision.

The use of the agglutination reaction of the normal serum is the only laboratory method available to the vast majority of general practitioners. The reliance on this point is practically absolute among the large majority of physicians, and it is not curious when one refers to clinical literature and teachings.

The results seem to indicate the following:

1. The great variation of types of infecting micro-organisms.
2. Paratyphoid infections are probably endemic in Pennsylvania.
3. The importance of differentiating infections by types of the typho-colon groups.



4. The importance of the infection of water-supplies from the fecal discharges of domestic animals as well as of human beings.

5. The suggestion that a mixed vaccine is of more importance than one of *B. typhosus* only.

I desire to express my very great appreciation to Dr. Samuel G. Dixon, Commissioner of Health, for this opportunity; to Dr. Herbert Fox, J. B. Rucker, Jr., and Alexander Garcia for their laborious work, and to many physicians in Iselin, Bethlehem and Coatesville, who made it possible to study their patients.

## THE RESISTANCE OF RETICULATED ERYTHROCYTES \*

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PHILADELPHIA

Ever since the recognition of the many interesting phenomena revealed by the application of the method of vital staining to the erythrocyte, hematologists have been endeavoring to derive from these findings facts of practical importance. As each discovery was announced some significance was attached to it, and the hope was raised that thereby some light was to be shed on the etiology of blood-diseases or on the vexed question of destruction and regeneration of the blood. It is now almost twenty years since the erythrocyte began to be studied by this means, and during this time almost twenty varieties of intra-corpuscular phenomena have been demonstrated and a most voluminous literature has collected. The majority of these findings, however, have not as yet been shown to be of any significance, many have been explained as merely artefacts and only a few are now considered worthy of mention in the newer works on hematology. Also, unfortunately, much of the work has been described without correlation with previous reports, and this has led in some instances to the same finding being credited to different discoverers in different countries, with, therefore, more than one name attached to it and often with the application of more than one significance. It is remarkable, however, how little discussion of this subject appears in English or American medical literature, whereas French, German and Italian articles are numerous. For these reasons the main points of the general subject are here reviewed somewhat in detail.

The one type of erythrocytic alteration demonstrable by vital staining which has been universally accepted as of importance is known variously as "skeined" or reticulated erythrocytes (*Hématies granuleuses* of the French), the basic staining substance of which is often spoken of as the Rosin-Demel substance, after two of the early workers in this field, or as *Fadenförmige substanz* or *Filarmasse* of the Germans, *Substantia granulofilamentosa*, etc. Briefly, it may be described as an intra-erythrocytic phenomenon, demonstrable only by vital staining with a basic dye, occurring in a small percentage (0.5 per cent.) of the erythrocytes of normal blood and in much larger percentages in many pathologic conditions. Its distinctive structure is suggested by its various names and is quite variable; sometimes being only a few granules connected by fine threads,

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\*Submitted for publication May 14, 1913.

at other times a net-work of threads with many granules, and again a thick ball-like tangle of threads. Its appearance is always characteristic and it cannot be confused with any of the other basophilic changes in the erythrocytes. It may be demonstrated either in wet vitally-stained preparations or in vitally-stained preparations which have later been fixed and which may be counterstained with Giemsa's or Wright's stain.

Although at first some investigators believed that this basophilic substance of the reticulated erythrocytes was an indication of disease of the cell and others that it was due to beginning degeneration, the more recent opinion is almost unanimous that these skeins are only to be found in young erythrocytes and that an increase in the number of reticulated cells is evidence of unusual activity in blood production. It is also generally accepted that they are analogous to the polychromatophilic cells of fixed preparations, but their relation to the common basic granulations of fixed preparations is still a much mooted point.

A simple enumeration of the reticulated cells in various types of disease has been repeatedly made and with quite uniform findings, but with results of little practical significance. If it is true that these cells represent young cells and that their number is an index of blood production, several interesting problems present themselves. The two most obvious ones to which this method might be applied have, however, been but little studied. In the first place the percentage of skeined forms might be employed as a means of estimating the therapeutic activity of hematinic medication or other therapeutic measures and this we are at present undertaking in connection with the use of benzol in leukemia. The second possible application is to the problems concerned with the attempt to interpret, as characteristic of young cells, the other alterations in the properties of the erythrocytes associated with the increase of reticulated forms. Of these alterations the one in which we have been interested is the increased resistance of the erythrocytes, under given conditions, to sodium chlorid hemolysis and the relation of this increased resistance to the percentage of reticulated forms present. In other words, does the reticulated erythrocytes show increased or decreased fragility as compared with the unreticulated cells of the same blood, and does this relationship exist in all conditions of altered hemolysis? This problem is also concerned with the degree of hemolysis produced by the gradations between the maximum and minimum hemolysis and the type of cells affected. By maximum resistance is meant that concentration of sodium chlorid in which complete hemolysis occurs, while by minimum resistance is meant the concentration of sodium chlorid solution in which the least discoloration of the fluid is preceptible. What degree of hemolysis is produced by the solutions of intermediate strength is known only in an indirect way through the percentage estimation of the hemoglobin con-

tent of the hemolytic solution, as compared with the content of a similar amount of solution containing the products of complete hemolysis of the same amount of corpuscles. This method was introduced by Theobald Smith and Brown<sup>1</sup> and later used by Gay.<sup>2</sup>

A number of authors have assumed from the coincident alteration in resistance and increase in reticulated forms that the former was dependent on the latter. Such assumptions have been made in almost all conditions that show both these alterations from the normal, and so theoretically has this been done that both increase and decrease of erythrocytic fragility have been attributed to an increased presence of young skinned cells.

The increase of skinned forms in the newly-born infant is very marked and may reach 20 per cent. Cathala and Daunay<sup>3</sup> have attempted to show that the physiological increase of skeins seen in the embryo and during the first few days of life is the explanation of the decrease of resistance of red cells observed by them at this time, but they conclude that the two findings do not alter in proportion to each other. On the other hand, some observers have noted an increased resistance of the fetal erythrocytes which continues a short time after birth.

The list of pathological conditions in which an increased number of reticulated forms has been described is long, but many of these conditions can be grouped together if the underlying causative anemia is recognized. Briefly, it may be said that all anemias, except those which have become aplastic through chronicity or lack of regenerative power of the bone-marrow, show an increased percentage of skinned forms. Also it may be said that in almost every anemia there is an alteration in the resistance of the erythrocytes to sodium chlorid hemolysis and this alteration is almost constantly an increase of resistance more or less proportionate to the degree of anemia. Here again authors have assumed a relationship between the two phenomena, and especially so in secondary anemia, where both the resistance and the number of reticulated cells are, as a rule, increased. Pernicious anemia shows a similar state of affairs, and in this disease the presence of skeins is of importance since their disappearance is a strong argument, as has been emphasized by Vaquez<sup>4</sup> for the diagnosis of aplastic change of the bone-marrow.

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1. Smith, Theobald, and Brown, H. R.: The Resistance of the Red Blood Corpuscles of the Horse to Salt Solutions of Different Tonicities before and after Repeated Withdrawals of Blood. *Jour. Med. Research*, 1906, xv, 425.

2. Gay, Frederick P.: The Function of Tonicity in Human Isohemagglutination. *Jour. Med. Research*, 1907-8, xvii, 321.

3. Cathala, V., and Daunay, R.: Les hématies granuleuses, la résistance globulaire à la naissance et pendant les premiers jours. *Compt. Soc. de biol.*, 1908, lxiv, 801.

4. Vaquez, M. H.: Anémie et rénovation sanguine. *Bull. et mém. Soc. méd. d. hôp. de Paris*, 1907, xxiv, 1532.



The history of the development of this point of view is interesting. In a case reported by Widal, Abrami and Brulé,<sup>5</sup> the patient suffered from an azotemic nephritis with intense anemia which resembled the aplastic type, but many skeins were present in the blood and no myelocytes; the fragility was normal. At autopsy the bone-marrow gave every appearance of activity. At the same time these investigators studied the effect of bleeding in animals and produced thereby an anemia with cells showing an increased number of skeins and an increased resistance to hemolysis. At this result they were greatly surprised, for at the time the French school believed the "*hematies granuleuses*" to be diseased corpuscles and to have a lowered resistance. They also, in another series<sup>6</sup> of experiments, produced an hemolytic anemia and observed that the resistance became lowered before any increase of reticulated cells appeared, and that the two phenomena did not coincide; also, they observed that similar conditions may be seen in human cases; that is, that either phenomenon may occur without the other. The opinion of the French school that the reticulated forms are associated with decreased resistance to hemolysis was due to the observations of Chauffard and Fiessinger, who, while looking for some disease of the erythrocyte that would help to explain the increased fragility seen in congenital hemolytic jaundice, found that the blood in such cases showed an enormous number of *hematies granuleuses*. To these they attributed the decreased resistance and considered such cells as diseased corpuscles. Their finding has been frequently confirmed, but not their interpretation. No one now believes the reticulation of the erythrocytes to be an evidence of cellular disease or of autolysis as was believed by Feuillé. It is in these cases of so-called congenital or familial hemolytic icterus that the highest counts of skinned corpuscles are seen, sometimes as many as 65 per cent. of the erythrocytes showing this phenomenon.

Heinz,<sup>7</sup> in 1890, was the first to observe changes in the erythrocytes following the experimental injection of pyroëdin, or phenyl-acetylhydrazin, a substance closely related to phenylhydrazine which is now more frequently used in laboratory work. The alterations which he observed and described are generally called, after him, the "Heinz bodies." These bodies he demonstrated by vital staining with a fluid prepared by saturating a 0.6 per cent. sodium chlorid solution with methyl violet. Their appearance is that of small blue staining knobs at the periphery of the erythrocytes, one or two to a cell, and of about the

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5. Widal, F., Abrami, P., and Brulé, M.: Anémie grave mortelle chez une brightique azotémique. Bull. et mém. Soc. méd. d. hôp. de Paris, 1907, xxiv, 1427.

6. Widal, F., Abrami, P., and Brulé, M.: Pluralité d'origine des ictères hémolytiques. Bull. et mém. d. Soc. méd. d. hôp. de Paris, 1907, xxiv, 1354.

7. Heinz, R.: Morphologische Veränderungen der rothen Blutkörperchen durch Gifte. Virchows Arch. f. path. Anat., 1890, exxii, 112.

size of a blood-platelet which, indeed, many authors have believed them to be. Heinz, however, considered them to be particles of necrotic protoplasm, and this view is most generally accepted. They have been found also to correspond to the "inclusion bodies" of Ehrlich,<sup>8</sup> which are seen in fixed preparations. Many further investigations were carried out in experimental phenylhydrazine poisoning, and it was discovered that in addition to the production of Heinz bodies by this blood-poison there also occurred a great increase in the number of reticulated cells. Later, Morawitz and Pratt<sup>9</sup> announced that in experimental phenylhydrazine poisoning in the rabbit there occurred also a very marked increase of the resistance of the erythrocytes. This increase is so great that after the fourth daily injection of 0.01 gm. phenylhydrazin chlorid the erythrocytes are not completely hemolyzed even by distilled water. This increase of resistance holds also for all types of hemolysins and is associated with the intense anemia which results from the injections of the poisons. A further phenomenon in the blood in such toxic anemias was described by Itami and Pratt<sup>10</sup> as an increase of stroma which they found went parallel to the increase of resistance. Rosenthal,<sup>11</sup> however, found no such relationship, and believes that the increase of stroma should perhaps be considered as analogous to cloudy swelling. Suzuki<sup>12</sup> made an exhaustive study of the blood in phenylhydrazine-poisoned animals, and concludes that the heightened resistance is due not so much to a diffuse "*pachydermie*" as to the formation and presence of Heinz bodies. Cells containing Heinz bodies he believes to be highly resistant in all test solutions and it is these cells which become larger, more voluminous and more irregularly shaped the longer the poisoning lasts. He came to this general conclusion as a result of studying the sediment of blood partially hemolyzed with 0.6 per cent. saponin. By staining this sediment he obtained little else than an enormous mass of Heinz bodies with only an occasional shadow corpuscle. In the sediment of a 10 per cent. saponin solution he found nothing but well-preserved Heinz bodies. In a similar way, Hartwick<sup>13</sup> prepared his so-called pure culture of Heinz bodies for chemical study. It does not seem probable that the persistence of these

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8. Ehrlich, P.: Kongress für innere Medizin, Leipzig, 1892.

9. Morawitz, P., and Pratt, J.: Einige Beobachtungen bei experimentellen Anämien. Münch. med. Wehnschr., 1908, iv, 1817.

10. Itami, S., and Pratt, J.: Ueber Veränderungen der Resistenz und der Stromata roter Blutkörperchen bei experimentellen Anämien. Biochem. Ztschr., 1909, xviii, 302.

11. Rosenthal, F.: Die sogenannte Pachydermie der Erythrozyten bei Phenylhydrazinämie. Folia haematol., 1910, Arch., x, 253.

12. Suzuki, T.: Weitere Beiträge zur Kenntnis der Erythrozytenveränderungen bei Pyrodivergiftung. Folia haematol., 1912, Arch., xiii, 225.

13. Hartwick, W.: Weitere Beiträge zur Kenntnis der Heinzschen Vergiftungskörper (Ehrlichsche hämeglobinamische Innenkörper). Folia haematol., 1912, Arch., xiii, 257.

bodies explains the apparent increased resistance of the erythrocytes since the Heinz body is but a small portion of the cell and its persistence would in no way interfere with the hemolysis of the remainder of the erythrocyte.

To summarize: In experimental work, a relationship has been assumed between the increased resistance and the increase of intra-erythrocytic phenomena, just as has been done in clinical types of anemia; but this has never been proved.

Direct attempts have been made in several ways to estimate how many and which cells are hemolyzed by the various degrees of hemolysis. Snapper,<sup>14</sup> apparently unfamiliar with the work of Smith and Brown,<sup>1</sup> and Gay,<sup>2</sup> attempted to reach some conclusion by estimating the amount of hemoglobin in solution in the various percentages of salt solution and reached the conclusion, on what grounds it is difficult to see, that the increased resistance in experimental anemia is due to young erythrocytes, and that it is proportionate to the degree of regeneration. In a rough way he attempts to draw numerical relations, but fails to reach any positive finding. Smith and Brown concluded that the facts concerning hemolysis do not permit the hypothesis that the younger erythrocytes, as a group, are either less or more resistant than the older cells, and that the theory most in harmony with the facts disavows any relationship between age of corpuscles and resistance to salt solutions. Nor could they find any close correspondence between size of corpuscles and the resistance.

In a manner similar to that employed by Suzuki in his studies of Heinz bodies, a few investigations have been made of the sediment, in tubes of partial hemolysis, to determine the presence and percentage of reticulated forms. Among the first reports of this nature was that of Chauffard and Fiessinger,<sup>15</sup> who, in 1907, studied the reticulated corpuscles at various stages of hemolysis and, contrary to their expectations, did not find them disappearing more quickly or more readily than the normal erythrocytes; in fact, the skeined forms seemed to persist longer. Fiessinger<sup>16</sup> later confirmed these findings. Sabrazes and Leuret<sup>17</sup> also made observations in a similar manner — on the blood of icteric infants which sometimes contained as many as 18 per cent. of skeined forms — and found that the reticulated cells did show increased resistance.

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14. Snapper, J.: Vergleichende Untersuchungen über junge und alte rote Blutkörperchen. *Biochem Ztschr.*, 1912, xliii, 256.

15. Chauffard, A., and Fiessinger, N.: Recherches experimentales sur les rapports entre l'hémolyse et les hématies granuleuses. *Bull. et mém. Soc. méd. d. Hôp. de Paris*, 1907, xxiv, 1367.

16. Fiessinger N.: Congrès de méd. de Lyon, October, 1911.

17. Sabrazes, J., and Leuret, E.: Hématies granuleuses et polychromatophilie dans l'ictère des nouveau-nés. *Compt. rend. Soc. de biol.*, 1908, lxiv, 423.

The most careful work in this respect has been done by Ravenna,<sup>18</sup> who estimated the fragility according to Hamburger's technic and after staining the sediment made careful counts of the percentage of skeined forms found. These counts showed a marked increase over the percentage found in the unhemolyzed blood. The counts were made both from fixed smears of the sediment after staining and from fresh-stained preparations by the use of a counting pipet and chamber. By these two methods very nearly the same figures were obtained. Counts were made from the sediment in three tubes, which he designated  $R_1$ ,  $R_2$ ,  $R_3$ , corresponding to the maximal, mediate and minimal degrees of hemolysis; for example, one blood showed the following figures: In the sediment from the tube containing 0.5 per cent. NaCl solution were found 3 per cent. skeined forms, while from the tube containing 0.32 per cent. NaCl solution the sediment revealed 86.9 per cent. of skeined corpuscles. He obtained his highest increase by means of the pipet and counting chamber; in one blood the isotonic tube showed 0.37 per cent. of skeined forms, while a strongly hypotonic tube showed 71.9 per cent. In all these experiments the stain used was brilliant cresyl-blue, and this was added directly to the hemolytic fluid, thus probably increasing its hemolytic power a little. Ravenna concludes as the result of his findings that the corpuscle showing reticulation when vitally stained is the most resistant of all to hypotonic salt solutions, whereas the one showing granulations in fixed preparations is much less resistant, and this is explained, since the latter is a degenerative form, whereas the former is a young cell offering evidence of regeneration.

Luzzato and Ravenna,<sup>19</sup> a year later, used the same method in studying the autolysis of erythrocytes and found that the skeined forms are more resistant than many of the unskeined, but nevertheless when the skeined forms have all disappeared there are still a moderate number of unskeined forms not hemolyzed.

One of the difficulties in making accurate estimations in this work is the finding of large numbers of shadow corpuscles, even in the upper stages of hemolysis; and whereas these unskeined shadows are often overlooked, it is impossible to miss the skeined shadows with their bundle of dark-blue threads. This fact is probably very important and may readily account for some of the results reported by careless investigators, or by those who believe that such forms should be counted as unhemolyzed cells. Wladimir Alexieff<sup>20</sup> discussed this theoretically and described the

18. Ravenna, F.: Sulla resistenza delle emazie granulose nelle soluzioni choro-sodiche. *Lavori e riviste di chimica e microscopia clinica*, 1909, i, 325.

19. Luzzatto, A. M., and Ravenna, F.: Sue fenomeni di autolisi nelle emazie granulose. *Folia clinica, chemica et microscopica*, 1910-11, iii, 53.

20. Alexieff, W.: Differences de structure et de signification des hématies granuleuses et des érythrocytes ponctués. *Arch. d. mal. du coeur, etc.*, 1911, iv, 552.



phenomenon which he calls hemoglobinolysis, and which he distinguished from hemolysis. He believes hemoglobinolysis occurs more readily in skeined forms, and he advances in explanation that these forms have a liquid protoplasm which more readily gives up its hemoglobin. It is to hemoglobinolysis of these cells that he attributes the jaundice of congenital icterus and the prolonged stage of partial hemolysis seen in many hemolytic anemias. These hemoglobinolyzed forms thus persist with no further evidence of hemolysis and enter largely into the counts. As a result of an exhaustive study of the literature concerning reticulated corpuscles, only a small part of which is here briefly abstracted, one gets the general impression that the reticulated erythrocytes are more resistant to hemolytic agents than the unreticulated ones, and that this is explicable by their being young forms. It is, however, quickly discovered that few have made any careful attempts to investigate the matter and that except in Ravenna's work scarcely any figures are quoted, and only very general statements are made.

We have had occasion in connection with our study of reticulated erythrocytes to attempt to demonstrate an associated increased resistance, but we have not been able to do so with any degree of constancy. In all our experiments, rabbits were employed and we have studied their blood, both before and after various degrees of anemia had been produced by repeated injections of phenylhydrazine hydrochlorid. In the normal blood, the skeins were constantly present in less than 2 per cent. of the cells, and the sediment of tubes with varying degrees of hemolysis failed to show an increased percentage of these forms. In the anemic blood of the experimental animals, where the number of skeins was large (60 to 70 per cent.), we quite often obtained a moderate increase of percentage of reticulated forms after hemolysis, but with no constancy, and never were we able to demonstrate any such enormous changes as those reported by Ravenna. In these experiments we routinely used brilliant cresyl-blue as the vital stain, although at times experimenting with other basic stains, and we applied this stain according to both methods used by Ravenna and also according to several minor modifications, but without effect. In studying the hemolysis in the rabbits poisoned with phenylhydrazine it was found that erythrocytic resistance was often so increased that a very low percentage of salt solution had to be employed to produce marked hemolysis, but no marked increase of skeins in the sediment was ever seen. Counts made from each tube of a series varying by intervals of 0.05 per cent. NaCl from above the point of beginning hemolysis to complete hemolysis often showed only the slightest and inconstant variations. At the lower end of such a series the percentage of shadow forms containing skeins or of skeins with no remnant of corpuscles around them would often appear enormous, but it is certainly incorrect to speak of

such isolated skeins, or even of skeins in shadows as being cells more resistant than the others. The isolated skein is no more truly an evidence of resistance of the cell than the Heinz body. Without going into our work any further it can be stated that by using all the means at our disposal we were unable to demonstrate any increase of resistance of the reticulated erythrocytes to hypotonic salt solution. Nor does it seem very reasonable that there should be any such increase when one considers in what conditions an increase of skeined forms are seen. It is true that in secondary anemias both resistance and skeins are increased, but this is not always the case in pernicious anemia; while in congenital icterus, the condition in which the number of skeins is most marked, not only does the increase of resistance fail, but a true decrease of resistance is demonstrable. Similarly there are conditions in which we see an alteration in one or the other, that is, an alteration of the number of the skeins or an alteration in the fragility of the erythrocytes, without any change from the normal in the other characteristic of the cells. It seems probable, therefore, that the reticulated erythrocytes show no constant increase or decrease of fragility as compared with the unreticulated erythrocytes of the same blood.

Theoretically, it may be advanced that as the skeined forms are the younger cells they will in a more marked degree show, or will be the first to show, the alteration of resistance which the erythrocytes are undergoing in response to some stimulus; but whatever resistance they possess as reticulated forms will probably remain the same when they have become mature unreticulated forms.

#### SUMMARY

1. Of the intra-erythrocytic phenomena demonstrated by vital staining, that one known as reticulation of the erythrocytes is most important.
2. Reticulation of the erythrocyte is an evidence of youth of the cell.
3. A study of the literature of this subject leads to the belief that reticulated erythrocytes show greater resistance to hemolytic agents than do unreticulated forms. No consistent satisfactory proof of this has, however, been presented.
4. Our own experiments have failed to demonstrate any constant difference between the resistance of the reticulated and non-reticulated erythrocytes of rabbit's blood under normal and experimental conditions.

## SIMPLIFIED METHODS FOR QUANTITATIVE ESTIMATION OF CHLORIDS IN THE URINE \*

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The process generally followed to determine the amount of chlorids in the urine is precise. It is, nevertheless, complicated and time-consuming, even for the operator in the clinical laboratory. In addition, requiring the use of a buret, volumetric flask, volumetric pipets, filters and porcelain dishes, it demands a quantity of calibrated apparatus, which makes the method almost inaccessible to the general practitioner. The need of a simpler procedure, by which the usefulness of chlorid estimations might be made general, was recognized in Europe some years ago. Achard and Thomas,<sup>1</sup> in France, and H. Strauss,<sup>2</sup> in Germany, have introduced short methods comparable to the technic of albumin determinations by the Esbach tube, which in their opinion greatly facilitate chlorid estimations without sacrificing the degree of accuracy necessary for the diagnostic value of these analyses. Little use, however, seems to have been made of their work, and no references to the repetition or confirmation of their results are available. In order, therefore, to determine whether quantitative chlorid determinations can be made by methods which promise much in convenience, while losing little in precision, I have carried out, under the direction of Dr. Guthrie, the following studies of the methods and instruments of the authors mentioned above.

The two chief processes in use for the quantitative estimation of the chlorids in the urine are the Volhard<sup>3</sup> method and the method of Mohr.<sup>4</sup> On the former, the simplified method of Strauss is based; on the latter, the method of Achard and Thomas. The former of these will be described first, leaving the latter to be considered in the description of the work of Achard and Thomas.

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\* Submitted for publication May 6, 1913.

1. Achard, Ch., and Thomas, L.: *Bull. et mém. Soc. méd. d. hôp. de Paris*, 1912, xix, 596.

2. Strauss, H.: *Praktische Winke für die chlorarme Ernährung*, Berlin, 1910.

3. Volhard: *Jour. f. prakt. Chem.*, 1874, ix, 217.

4. Mohr: *Lehrbuch der Titrimethode.*, 1862, p. 317.

## FILTRATION TO REMOVE SILVER CHLORID UNNECESSARY

The Volhard<sup>3</sup> method, as modified by Drechsel<sup>5</sup> and applied to the urine by Falk<sup>6</sup> and Arnold,<sup>7</sup> consists in the precipitation of the chlorids by an excess of a standardized solution of silver nitrate, the removal of the silver chlorid by filtration and the titration of the excess of silver nitrate with ammonium thiocyanate, using an iron salt as an indicator. This method, modified only as regards the strength of the solutions employed, later was applied to gastric and urinary analysis by Lüttke and Martius.<sup>8</sup> The Lüttke-Martius method is the one used in this laboratory and has been the one employed in these experiments. While this method is accurate, it demands a considerable amount of calibrated apparatus, and requires more than thirty minutes for duplicate determinations. The original Volhard method, however, could be carried out more rapidly than this, as it did not include the removal of the silver chlorid by filtration, the titration with ammonium thiocyanate being done in its presence. In using this method, Drechsel,<sup>5</sup> and more recently Rosanoff and Hill,<sup>9</sup> found difficulty with the end-point, due, they thought, to a reaction between the silver chlorid and the ammonium thiocyanate. To obviate this, Drechsel introduced the step of filtration, which has since been incorporated in this method and its modification by Lüttke and Martius. As the omission of the removal of the silver chlorid is one of the means employed by Strauss in simplifying this technic, it becomes important to determine whether this step has any influence on the results of the titration. Volhard<sup>10</sup> subsequently showed the error in Drechsel's criticism, and Goodall,<sup>11</sup> recently, in a study of sixty urines of widely varying chlorid content, has found that the original Volhard is as accurate as the Volhard modified by the filtration step. Harvey<sup>12</sup> has reached a similar conclusion from numerous experiments on the reaction between these solutions. He has found that in the Volhard method the precipitated silver chlorid does not react with the ammonium thiocyanate while an excess of silver nitrate is present; and further, that after the excess of silver has been removed, the reaction between the silver chlorid and the thiocyanate is markedly retarded by the presence, first, of the ferric salt, and secondly, by the excess of nitric acid in which the silver nitrate is dissolved. To verify this point, I made the determinations shown in Table 1.

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5. Drechsel, E.: *Jour. f. prakt. Chem.*, 1877, 191.

6. Falk, A.: *Berichte d. deutsch. chem. Gesellsch.*, 1875, viii, 12.

7. Arnold, C.: *Arch. f. d. ges. Physiol.*, 1885, xxxv, 541.

8. Lüttke and Martius, F.: *Die Magensaure des Menschen*, Stuttgart, 1892. (Quoted by Strauss and others.)

9. Rosanoff and Hill: *Jour. Am. Chem. Soc.*, 1907, xxix, 269.

10. Volhard: *Liebig's Ann.*, 1877, cxc, 1.

11. Goodall, H. W.: *Boston Med. and Surg. Jour.*, 1909, clx, 304.

12. Harvey, S. C.: *THE ARCHIVES INT. MED.*, 1910, vi, 12.



TABLE 1.—CHLORID DETERMINATIONS BY LÜTTKE-MARTIUS METHOD

Urine	AgCl Removed by Filtration		AgCl Present		Difference in Grams
	Gm. NaCl in 100 c.c.	Average	Gm. NaCl in 100 c.c.	Average	
I .....	0.67860	.....	0.67860	.....	.....
	0.67860	0.67860	0.678015	0.678307	0.00293
II .....	0.3888025	.....	0.390195	.....	0.0013925
III .....	0.8541	.....	0.857025	.....	0.002925

The results summarized in Table 1 show that filtration to remove the silver chlorid is not necessary under the conditions outlined above. As will be seen later, the factors in the analysis by the Strauss method are exactly similar to these.

#### LÜTTKE-MARTIUS METHOD

As the Volhard, and therefore the Lüttke-Martius, method has been proved accurate, the results obtained by the Lüttke-Martius procedure have been taken as the standard for gauging the accuracy of the other methods tested in these studies. This method, differing from its prototype only in the strength of the solution employed, is based on the precipitation of chlorids by silver nitrate, and the titration of the excess of silver nitrate by ammonium thiocyanate, using an iron salt as an indicator. Two standardized solutions are required:

1. N/10  $\text{AgNO}_3$ —an acid solution containing iron ammonium alum. One cubic centimeter of this standardized solution will precipitate 0.00585 gm. NaCl.
2. N/10  $\text{NH}_4\text{SCN}$ —containing 7.6 gms. of ammonium thiocyanate to the liter of solution.

The preparation of these standard solutions will be described in detail in the consideration of the Strauss method.

To make a determination by this method, the procedure is as follows:

By means of a volumetric pipet, 10 c.c. of urine are placed in a 100 c.c. volumetric flask. To this are added 20 c.c. of N/10  $\text{AgNO}_3$  from a volumetric pipet. This mixture is shaken gently and allowed to stand for five minutes, until the precipitation of chlorids is complete. Distilled water is then poured into the flask, filling it to the 100 mark. The flask is stoppered, shaken well, and allowed to stand for a few minutes. If a reddish color appears in the mixture, as is nearly always the case with highly colored febrile urines, this is cleared by adding three or four drops of a solution of potassium permanganate. The mixture is filtered to remove the silver chlorid. Then, 50 c.c. of the filtrate are put into an evaporating dish by means of a volumetric pipet, and into this N/10  $\text{NH}_4\text{SCN}$  is allowed to run, drop by drop, from a buret. At first, a brown color appears, due to the formation of ferricyanate. But in the presence of an excess of silver nitrate this immediately disappears, giving place to a bluish white precipitate of silver cyanate. The titration is continued until a permanent reddish brown color appears in the fluid. This color, due to the formation of

ferrieyanate, occurs permanently when all of the silver has been precipitated. The reading of the buret at this point denotes the excess of silver in 50 c.c., or half of the amount of fluid in the flask. This figure, therefore, must be multiplied by two in order to obtain the amount of the silver in excess in the total volume of the mixture. From this the chlorid content is calculated as follows: For example:

Buret reading shows 6 c.c. N/10  $\text{NH}_4\text{SCN}$  used.

Therefore  $6 \times 2 = 12 =$  excess of silver in total mixture. Then 20 c.c. N/10  $\text{AgNO}_3$  less 12 = 8 = c.c. of silver solution required to precipitate all chlorids in 10 c.c. of urine, and  $8 \times 0.00585 = 0.0468 =$  gm. of NaCl in 10 c.c. urine. If the total output of the urine examined were 1 liter, then the total chlorids would be 4.68 gm. per 1,000 c.c.

The amount of silver here employed is capable of indicating at most 11.7 grams of chlorids per liter. Urine, found by a preliminary test to contain more chlorids than this, must be diluted, or a larger quantity of silver nitrate may be added, before the analysis can be made.



Fig. 1.—Strauss chloridometer.

#### THE STRAUSS METHOD

The chief simplification of this process has been introduced by Prof. H. Strauss<sup>2</sup> of Berlin. He has condensed into a single tube<sup>13</sup> all of the necessary calibrated apparatus, and claims simplicity together with sufficient clinical accuracy for his method of chlorid determinations. The author has not formally published the results which he has obtained by the use of this instrument, but in a letter has written me that he has been using it for some time with satisfaction. The method is simply the Lüttke-Martius process done in one piece of apparatus. The filtration

13. The Strauss Chloridometer is manufactured by Paul Altmann of Berlin. Price 2.50 marks.

to remove the precipitate of silver chlorid—a step already shown to be unnecessary—is omitted.

The Strauss instrument, shown in Figure 1, is a glass tube 20 cm. long and 2 cm. in diameter. Its lower end is broadened into a base, too small, however, to steady effectually the long cylinder. The upper end is fitted with a ground-glass stopper. Its barrel is calibrated with the marks shown in the figure, to indicate the volumes of silver nitrate solution and of urine to be placed in the tube. Above the mark for the urine, the tube bears a scale reading in 0.5 gm. from 11 gm. to 0.5 gm. These scale marks indicate grams per liter. Urine containing more than 11 gm. of chlorids per liter must be diluted before being analyzed by this method.

For the purpose of the analysis, two standardized solutions<sup>14</sup> are required:  
1. N/10 AgNO<sub>3</sub> (Lüttke-Martius' silver solution).

This solution contains 16.966 gm. of silver nitrate to the liter. 1 c.c. of this solution will precipitate 0.00585 gm. NaCl. It is prepared as follows:

- 17.5 gm. AgNO<sub>3</sub>
- 900 c.c. HNO<sub>3</sub> (25 per cent.)
- 50 c.c. iron ammonium alum, a cold saturated solution, which is about a 10 per cent solution.
- Distilled H<sub>2</sub>O to 1,000 c.c.

An excess of silver nitrate is used because the salt is somewhat hygroscopic and hence the true amount of silver nitrate is not indicated by the weight of the substance. This solution is standardized in the usual way by titration against known normal hydrochloric acid, the iron salt in the solution acting as an indicator. Thus, 20 c.c. of the silver nitrate solution are added to 20 c.c. of known tenth normal hydrochloric acid. A solution of ammonium thiocyanate is added to this, and the silver solution adjusted until the end-point, or brownish red color, is given on the addition of a very small drop of the thiocyanate. The solution, as made up, will have to be diluted in the process of standardization. This dilution is done by adding a special fluid containing seven parts of distilled water, 2.5 parts of concentrated nitric acid, and 0.5 parts of a 10 per cent. solution of iron ammonium alum. In this way, as has been shown, the factors necessary for a sharp end-point are provided. When kept in a brown bottle, away from the light, this solution remains constant in strength for a long time.

- 2. N/20 NH<sub>4</sub>SCN (3.8 gm. NH<sub>4</sub>SCN per liter).

This solution is one-half the strength of the ammonium thiocyanate of the Lüttke-Martius method. Its greater dilution allows a scale of longer intervals to be marked on the tube. It is prepared by dissolving 4 gm. NH<sub>4</sub>SCN in 1,000 c.c. of distilled water. Then, by titration with the standardized solution of silver nitrate, in which the iron salt acts as an indicator, it is adjusted by dilution, with water or the addition of more of the thiocyanate, as indicated, so that

$$2 \text{ c.c. N/20 NH}_4\text{SCN} = 1 \text{ c.c. N/10 AgNO}_3.$$

In order to make a chlorid estimation with the Strauss instrument, N/10 AgNO<sub>3</sub> (Lüttke-Martius' silver solution) is placed in the tube until the bottom of the meniscus corresponds to the mark A. Urine is added until the mark U is reached. These fluids are mixed by gently inverting the tube several

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14. Prof. Strauss writes that he has been using such solutions as these prepared by Kahlbaum, and he has found them satisfactory. They are prepared also by pharmaceutical houses in this country.

times. Shaking, which causes the formation of bubbles, must be avoided. The tube is then allowed to stand for five minutes. If a reddish color develop in the mixture, as occurs with all but pale urines, 3 drops of a 10 per cent. solution of potassium permanganate are added. This decolorizes the fluid and the amount of potassium permanganate does not markedly raise the level of the fluid in the tube. In any case, allowance for the increase in the fluid may be made when the scale is read at the end of the titration. After five minutes,  $N/20 \text{ NH}_4\text{SCN}$  is run gradually into the tube by means of a buret, pipet, or through a siphon from a bottle of the solution on a shelf. After each addition of the ammonium thiocyanate, the tube is inverted several times gently. As in the case of the Lüttke-Martius' titration, the reddish brown color which appears at first, immediately disappears as long as an excess of silver is present. The addition of ammonium thiocyanate is continued until a definite, permanent reddish-brown color in the fluid is obtained. This end-point, with solutions made up as prescribed, is sharp and lasting. It may be appreciated most readily by holding the tube against a white background. It is well to obtain a good reddish-brown color, for a drop or so of the thiocyanate makes little difference in the scale readings, but a great deal of difference in the intensity of the color. When the titration is complete, the amount of the chlorids in *grams per liter* is read off from the scale.

Experiments to test the accuracy of these instruments, and of the method, were carried out along the following lines:

- (a) tests of the calibrations of the tube,
- (b) estimations of chlorids in aqueous solutions,
- (c) estimations of the chlorids in normal urine,
- (d) estimation of the chlorids in urines containing albumin.

In all titrations, results obtained by the Lüttke-Martius method, were taken as the absolute for comparison.

(a) *Calibration of Strauss Tubes.*—The volumes contained between the various marks on Strauss tubes should be as follows: From bottom of tube to the mark A, 10 c.c.; from A to U, 5 c.c.; from U to 11, 1.2 c.c.; and between every mark thereafter on the scale, 0.9 c.c. The calibration of the six tubes imported by this laboratory was tested by comparison with the readings of a standardized buret. By this test, none of the tubes was found to be perfectly accurate. In some the errors were greater than in others, a discrepancy of as much as 0.2 c.c. between the tube-volumes and the absolute scale being noted. As a rule, however, the A and U volumes were found to be in the proper relation. The tubes are put out uncorrected and unaccompanied by any guarantee of accuracy. These errors of workmanship, which, on the whole, are not very large, are therefore to be expected. They do not detract from the value of the tube as a clinical instrument.

(b) *Estimation of Chlorids in Aqueous Solutions.*—Solutions containing sodium chlorid in amounts corresponding to all of the gradations on the apparatus were analyzed. The results of these determinations with the six Strauss tubes are presented in Table 2.



TABLE 2.—ESTIMATION OF CHLORIDS IN AQUEOUS SOLUTION BY STRAUSS' METHOD COMPARED WITH LÜTTKE-MARTIUS' METHOD

Grams NaCl per liter by						
Lüttke-Martius'	Strauss					
Method	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
0.5 .....	0.5	0.3	0.3	0.5	0.3	0.6
1.228 .....	1.3	1.5	1.5	1.5	1.3	1.4
1.638 .....	1.8	2.05	2.	2.	1.8	2.
2.106 .....	2.1	2.1	2.5	2.1	2.1	1.9
2.574 .....	2.6	2.9	2.7	.....	2.7	3.
3.276 .....	3.5	3.4	3.5	3.5	3.5	3.2
3.627 .....	3.9	3.7	3.7	3.6	3.55	3.6
3.9953 .....	4.	4.05	4.05	4.05	4.	4.4
4.797 .....	4.75	4.75	5.	4.75	5.	4.75
5.0885 .....	4.9	5.1	5.05	5.02	5.1	5.
5.5867 .....	5.6	5.7	5.8	5.8	5.7	5.9
6.101 .....	6.05	6.1	6.	6.1	6.	6.
6.5227 .....	6.75	6.5	6.6	6.6	6.6	6.6
7.254 .....	7.1	7.5	7.5	7.5	7.2	7.1
7.605 .....	7.55	7.6	7.55	7.55	7.8	7.8
8.1607 .....	8.1	8.1	8.15	7.8	8.2	8.
8.5702 .....	8.6	8.7	8.7	8.6	8.5	8.8
9.0967 .....	9.4	9.5	9.3	9.1	9.4	9.5
9.5355 .....	9.55	9.8	9.55	9.5	9.5	10.
10.2667 .....	10.	10.2	10.5	10.6	10.2	10.5
10.764 .....	11.	10.7	11.	10.8	10.7	10.9
11.0565 .....	11.1	11.1	11.2	11.4	11.05	11.3
Max. difference from Lüttke-Martius .....	0.3033	0.403	0.394	0.3435	0.3033	0.426
Min. difference .....	0.	0.001	0.0145	0.	0.0047	0.0115
Average difference .....	0.1516	0.202	0.2042	0.1717	0.154	0.2187

It is shown by this table that the results obtained by the Strauss method are somewhat at variance with the Lüttke-Martius determinations and also exhibit discrepancies between the different tubes. The greatest variation from the absolute (Lüttke-Martius) standard for any amount of chlorids was 0.426 gm. per liter; the smallest was 0. The average of these variations was approximately 0.2 gm. per liter. In nearly all cases the tubes indicated amounts too high. These discrepancies must be attributed to the errors in the calibration of the tubes and to the experimental error in reading the buret. Readings of approximately equal accuracy were obtained from all regions of the scale.

(c) *Estimation of Chlorid in Normal Urine.*—Normal urines of varying chlorid content were analyzed by the Lüttke-Martius and Strauss methods. The results of these tests are summarized in Table 3.

The results of these titrations of the chlorids in urine, even more than those of chlorids in aqueous solution, exhibit the satisfactory accuracy of the Strauss method. In this series (Table 3), the largest discrepancy between the Strauss estimation and the Lüttke-Martius was 0.349 gm. per liter, and this occurred in analyzing a urine containing more than 11 gm. of chlorids per liter. This reading was an approximate one, as the fluid in the tube was below the last scale mark. Even with this, however, the average mean of error was 0.1343 gm. per liter. While this error would be very large in a purely chemical analysis, it is negligible in a clinical estimation. On these results, therefore, it may be concluded that the Strauss method applied to the normal urine is amply accurate to serve as a basis for clinical diagnosis.

TABLE 3.—STRAUSS TUBE ESTIMATION OF CHLORIDS OF NORMAL URINE

Method	Grams NaCl per Liter					
Lüttke-Martius <sup>1</sup> .....	2.0475	4.9725	6.903	7.722	9.716	11.349
Strauss—						
Tube 1 .....	2.05	4.8	6.9	7.8	9.5	11.5
Tube 2 .....	2	4.7	7	7.6	9.8	11.5
Tube 3 .....	2	.....	6.6	7.7	9.8	11.1
Tube 4 .....	1.9	.....	.....	7.6	9.6	11
Tube 5 .....	.....	5	7.05	7.6	9.7	11.5
Tube 6 .....	.....	5	.....	7.7	9.8	11.5
Max. difference .....	0.1475	0.2725	0.303	0.122	0.216	0.349
Min. difference .....	0.0035	0.0275	0.003	0.022	0.016	0.151
Mean difference .....	0.0755	0.15	0.153	0.072	0.1165	0.250

Several of the urines in the above series were so highly pigmented that their color interfered with the sharp appearance of the end-point. They were, however, readily decolorized by adding three or four drops of a 10 per cent. solution of potassium permanganate to the mixture in the tube a few minutes before titration. As a rule, the color which develops while the urine stands in contact with the strongly acid silver nitrate solution is made sufficiently pale by dilution from the subsequent addition of the ammonium thiocyanate. But even in normal urine this color may obscure the end-point. It is well, therefore, whenever working with a urine of a deep yellow color to decolorize the mixture in the tube by adding a few drops of the solution of potassium permanganate. Whatever increase in the level of the fluid in the tube is caused by this may be discounted in reading the scale. The advantage of a much more distinct end-point is gained by this step.

(d) *Estimation of Chlorids in Albuminous Urines.*—Although Arnold,<sup>7</sup> in estimating the chlorids in a variety of fluids—milk, blood-serum and urines containing albumin—showed that the presence of

protein in the fluid under analysis did not interfere with the accuracy of the Volhard method, it was considered well to repeat some of his work and to determine whether the Strauss procedure would be disturbed by the presence of protein. It is conceivable that some of the silver of the N/10 silver nitrate solution might become bound to the albumin, forming a silver albuminate, and thus be unavailable in the titration. To test this point solutions of chlorid-free protein of varying concentration were added to normal urine of known chlorid-content and of constant volume, and the resulting mixture was titrated as in the foregoing experiments. The salt-free protein was obtained from blood-serum by dialyzing the serum against changes of distilled water until the water around the dialyzing sac no longer gave a cloudiness with silver nitrate, and the protein in the sac began to precipitate. In this way, solutions of protein were prepared containing from 0.7 to 30 gm. of protein per liter (Tsuchiya). These solutions were shown to be salt-free by Lüttke-Martius determinations. When 10 c.c. of the protein solution were added to 20 c.c. N/10  $\text{AgNO}_3$ , and the mixture titrated for the excess of silver, the result was found to correspond exactly with what was obtained when those amounts of the standard solutions were titrated. The solutions balanced.

In these tests, however, a heavy yellowish precipitate was formed when the protein was added to the strongly acid silver solution. This precipitate interfered with the analysis only by its bulkiness, when more than 30 gm. of protein per liter were present, but it did not at all disturb the balanced reaction between the standardized silver nitrate and ammonium thiocyanate. The results of a series of titrations with urines containing proteins are given in Table 4.

From Table 4 it is evident that in the time in which the analysis is performed, none of the silver of the standardized solution becomes bound to the protein in such a manner as to render it unavailable for the titration. This table shows that the greatest discrepancy between the Lüttke-Martius and Strauss results was 0.343 gm. of chlorids per liter, and that this occurred only in the analysis of the urine containing 27 gm. of protein per 1,000 c.c. An error as large as this was to be expected from the previous experiments with protein-free urine. But as regards the precipitation of the silver in combination with the protein, the most significant fact is that chloridometry by the Lüttke-Martius method on equal quantities of the same urine, free from or containing protein, gives exactly equal results. Thus by these analyses of urine rendered artificially pathological under controllable conditions, it is shown that the presence of protein in amounts up to 27 gm. per liter does not interfere with the accuracy of the Strauss method.

TABLE 4.—STRAUSS TUBES WITH ALBUMINOUS URINES

URINE A			
Tests	25 c.c. Normal Urine. 50 c.c. Water	25 c.c. Same Urine 50 c.c. Protein A	Same Mixture Protein Removed. Vol. Not Restored
Heat and acetic ...	0	+	0
Tsuchiya .....	*	†0.7	*
Lüttke-Martius ...	‡4.446	4.446	4.5045
Strauss —			
Tube 1 .....	4.2	4.2	4.5
Tube 2 .....	4.5	4.3	4.5
URINE B			
Tests	50 c.c. Urine. 50 c.c. H <sub>2</sub> O	50 c.c. Same Urine 50 c.c. Protein B	Same Mixture Protein Removed. Vol. Not Restored
Heat and acetic ...	0	+	0
Tsuchiya .....	*	1.4	.....
Lüttke-Martius ...	5.7330	5.7330	5.9670
Strauss —			
Tube 1 .....	5.6	5.7	6
Tube 2 .....	5.7	5.6	6.05
URINE C			
Tests	25 c.c. Urine. 50 c.c. H <sub>2</sub> O	25 c.c. Urine 50 c.c. Protein C	Same Mixture Protein Removed. Vol. Not Restored
Heat and acetic ...	0	+	0
Tsuchiya .....	0	10	0
Lüttke-Martius ...	5.2065	5.265	5.3235
Strauss —			
Tube 1 .....	5.1	5.05	.....
Tube 2 .....	5.2	5.2	.....
URINE D			
Tests	10 c.c. Urine 90 c.c. H <sub>2</sub> O	10 c.c. Urine 90 c.c. Protein D	Same Mixture Protein Removed. Vol. Restored
Heat and acetic ...	0	+	0
Tsuchiya .....	.....	27	0
Lüttke-Martius ...	2.6910	2.6432	2.2113
Strauss —			
Tube 1 .....	2.8	2.3	2.3
Tube 2 .....	2.6	2.5	2.3

\* Not done.

† Chlorids expressed in grams per liter.

‡ Protein expressed in grams per liter.



A source of large error was introduced when the protein was removed from the urine by heat, acetic acid and filtration. The results of titration made after this manipulation are shown in the third column of Table 4. When the estimations are made on the filtrate not restored to the original volume, the amount of chlorids found is too high. But when the analyses are made on the filtrate restored to its original volume by the addition of water, the amounts are too low. The loss of water by evaporation, the carrying down or mechanical "locking up" of chlorids by the heavy precipitate of protein cannot be compensated for by dilution. It is better, therefore, to make the titrations directly in the presence of the protein contained in albuminous urine.

On the basis of the numerous tests described above it may be concluded that the Strauss method is an improvement on the usual methods for determining chlorids. From a clinical standpoint it is a simple, rapid and sufficiently accurate method for determining chlorids in both normal and pathological urine.

#### CHLORIDOMETRY IN GRADUATED CYLINDERS BY STRAUSS METHOD

Although the Strauss method is a simple and commendable one, it nevertheless retains some points of inaccessibility, as the instrument employed is of foreign manufacture, requiring importation and demanding a rather high price. It seemed well, therefore, to attempt a further simplification of the process. The standardized solutions, obviously, cannot be eliminated. Two of these are required in the Strauss method, but when a laboratory is able to prepare one standardized solution another can be made with only little additional difficulty. The instrument, however, can be simplified. In place of it, a simple graduated 50 c.c. cylinder may be used, and with such a "tube" and a scale for interpreting its results, sufficiently accurate chlorid estimations may be made, as is shown in Table 5.

In making determinations the same solutions and the same amounts of them were put into the cylinders as were used in the Strauss tubes. From the amounts of the various fluids employed, it is evident that when an end-point is reached in the titration, a definite volume of fluid is present in the tube. Thus, for example, it was seen by actual measurement that when the end-point was reached with 5 gm. per liter the Strauss tube contained 26.45 c.c. of total fluids. It follows, moreover, from the volume of silver nitrate solution, the urine and the ammonium thiocyanate, and from their numerical relationships, that the total volume of fluid present for any degree on the scale of chlorids may be calculated. In this way, total volume of fluid may be converted into grams of chlorids per liter, or *vice versa*. For this purpose the following formula has been derived:

Grams chlorids per liter =  $20.475 - 0.585 V$ . Eq. I where  $V$  is the total fluid present in the tube.

The formula is derived as follows:

Solutions placed in tube:

5 c.c. or 1/200 of a liter of urine

10 c.c.  $N/10 AgNO_3$ . Each c.c. of  $N/10 AgNO_3$  will precipitate 0.00585 gm. NaCl.

Sum of these solutions = 15 c.c.

$N/20 NH_4SCN$ , of which 2 c.c.  $N/20 NH_4SCN = 1$  c.c.  $N/10 AgNO_3$ .

$V$  = total fluid at end of titration.

$V - 15$  = amount of  $N/20 NH_4SCN$  used in titration.

$V - 15$  = Excess of  $N/10 AgNO_3$  present after precipitation of chlorids.

$$10 - \frac{2}{V - 15} = \text{amount of } N/10 AgNO_3 \text{ used in precipitation of chlorids.}$$

Then

$$\left[ 10 - \frac{2}{V - 15} \right] 0.00585 = \text{gm. NaCl in 5 c.c. urine.}$$

$$\left[ 10 - \frac{2}{V - 15} \right] 0.00585 \times 200 = \text{gm. NaCl in 1,000 c.c. urine.}$$

When simplified, this expression is

$$20.475 - 0.585 V = \text{gm. NaCl per liter of urine. Eq. I.}$$

Example:

Suppose total fluid at end of titration = 21.35 c.c.

Substituting in Equation I:

$$20.475 - 0.585 \times 21.35 = \text{gm. chlorids per liter.}$$

$$\therefore 7.98525 = \text{gm. chlorids per liter.}$$

In this case the Lüttke-Martius determination showed that the chlorids were 7.956 gm. per liter.

By a rearrangement of the terms of this formula, an expression is derived by which the amount of fluid present may be calculated from the number of grams of chlorids per liter in the solution analyzed. This formula would be useful in constructing or checking the calibration of an instrument:

$$V = \frac{20.475 \text{ gm. chlorid per liter}}{0.585} \quad \text{Equation II. where } V \text{ is the total fluid in the cylinder at the end of the titration.}$$

From Equation I a scale has been computed for use with the graduated cylinders, from which the numbers of grams of chlorids per liter of the urine, or solution tested, may be read off from the total amount of fluid present at the end of the titration. This scale has been calculated in 0.1 gm. NaCl per liter from 0 to 11.7 gm. per liter. Whereas the scale on the Strauss tube registers from 11 to 0.5 gm. per liter, this scale somewhat extends these limits. By means of it, a range of 1.2 gm. per liter beyond the limits of the Strauss scale is allowed for chlorid estima-

tions in the graduated cylinders. The calculated values of the scale correspond quite accurately to the amounts observed when testing volumetrically the calibrations of the Strauss tubes.

To test this method, a series of determinations were made with ordinary 50 c.c. cylinders chosen at random from the supply in the laboratory and with a certified cylinder of an accurate calibration. The graduations on the ordinary cylinders were for 1 c.c., extending over a tube length of 12.5 cm. The certified cylinder was graduated in 0.2 c.c., extending over a scale length of 25 cm. For these experiments the

SCALE OF CHLORIDOMETRY IN CYLINDERS

Chlorids Gm. per Liter	Volume	Gm.	Volume	Gm.	Volume	Gm.	Volume	Gm.	Volume	Gm.	Volume
0.0	35.000	2.0	31.58	4.0	28.16	6.0	24.74	8.0	21.32	10.0	17.90
0.1	34.829	2.1	31.409	4.1	27.999	6.1	24.569	8.1	21.159	10.1	17.739
0.2	34.671	2.2	31.238	4.2	27.828	6.2	24.398	8.2	20.988	10.2	17.568
0.3	34.487	2.3	31.060	4.3	27.657	6.3	24.227	8.3	20.717	10.3	17.397
0.4	34.316	2.4	30.896	4.4	27.486	6.4	24.056	8.4	20.546	10.4	17.126
0.5	34.128	2.5	30.72	4.5	27.30	6.5	23.88	8.5	20.48	10.5	17.04
0.6	33.971	2.6	30.549	4.6	27.129	6.6	23.709	8.6	20.309	10.6	16.869
0.7	33.803	2.7	30.378	4.7	26.958	6.7	23.538	8.7	20.138	10.7	16.698
0.8	33.632	2.8	30.207	4.8	26.787	6.8	23.367	8.8	19.967	10.8	16.527
0.9	33.461	2.9	30.036	4.9	26.616	6.9	23.196	8.9	19.796	10.9	16.356
1.0	33.29	3.0	29.87	5.0	26.45	7.0	23.03	9.0	19.625	11.0	16.19
1.1	33.119	3.1	29.699	5.1	26.279	7.1	22.869	9.1	19.454	11.1	15.919
1.2	32.948	3.2	29.528	5.2	26.108	7.2	22.698	9.2	19.283	11.2	15.748
1.3	32.771	3.3	29.357	5.3	25.937	7.3	22.527	9.3	19.112	11.3	15.577
1.4	32.606	3.4	29.186	5.4	25.766	7.4	22.356	9.4	18.941	11.4	15.406
1.5	32.435	3.5	29.025	5.5	25.605	7.5	22.175	9.5	18.755	11.5	15.33'
1.6	32.264	3.6	28.854	5.6	25.434	7.6	22.004	9.6	18.584	11.6	15.171
1.7	32.093	3.7	28.683	5.7	25.263	7.7	21.833	9.7	18.413	11.7	15.000
1.8	31.912	3.8	28.512	5.8	25.092	7.8	21.662	9.8	18.242	....	.....
1.9	31.741	3.9	28.341	5.9	24.921	7.9	21.491	9.9	18.071	....	.....

cylinders were closed with rubber stoppers. Titrations were made in these cylinders according to the Strauss method and the results compared with Lüttke-Martius and Strauss determinations on the same urines. The results of these analyses are shown in Table 5. In this table also are given the results of this method obtained when smaller cylinders (10 c.c.) were used. The significance of this will be described later.

Considering for the present only the results of titration in the 50 c.c. cylinders, it is seen from Table 5 that errors in chloridometry with the ordinary cylinders range from 0.149 to 0.271 gm. of chlorids per liter, while errors with the standardized cylinder are less, being from 0.021 to 0.12 gm. per liter of urine. The larger errors of the ordinary cylinders are explained by the errors found to be existent in their calibrations

when the supposed cubic content between the graduations was measured by means of a buret. The more accurate results with the certified cylinder were to be expected from the accuracy of its calibration and the

TABLE 5.—ESTIMATION OF CHLORIDS IN URINE BY STRAUSS METHOD IN GRADUATED CYLINDERS. RESULTS COMPARED TO STRAUSS TUBE AND LÜTTKE-MARTIUS DETERMINATIONS

Method	Tube and Lüttke-Martius Determinations				In Gm. per liter, Difference from Lüttke-Martius
	Gm. per liter Observed	Gm. per liter by Scale	Volume c.c. Observed	Volume c.c. by Scale	
Lüttke-Martius .....	0.2340	.....	.....	34.6	.....
Strauss tube .....	Meniscus above scale	.....	.....	.....	.....
Ordinary 50 c.c. cyl....	.....	0.5	34.1	.....	+0.266
Certified 50 c.c. cyl....	.....	0.15	34.75	.....	-0.084
Ordinary 10 c.c. cyl....	.....	0.75	6.75	.....	+0.516
Certified 10 c.c. cyl....	.....	0.25	6.85	.....	+0.016
Lüttke-Martius .....	0.7371	.....	.....	33.73	.....
Strauss tube 2.....	0.7	.....	33.6	.....	-0.0371
50 c.c. cyl. ord.....	.....	0.45	34.2	.....	-0.1871
50 c.c. cyl. cert.....	.....	0.8	33.6	.....	+0.0629
10 c.c. cyl. ord.....	.....	1.45	6.5	.....	+0.7189
10 c.c. cyl. cert.....	.....	0.75	6.75	.....	+0.0189
Lüttke-Martius .....	4.329	.....	.....	27.6	.....
Strauss tube 4.....	4.2	.....	27.88	.....	-0.129
50 c.c. cyl. ord.....	.....	4.6	27.1	.....	+0.271
50 c.c. cyl. cert.....	.....	4.35	27.5	.....	+0.021
10 c.c. cyl. ord.....	.....	5.1	5.25	.....	+0.771
10 c.c. cyl. cert.....	.....	4.25	5.55	.....	-0.079
Lüttke-Martius .....	8.5702	.....	.....	20.35	.....
Strauss tube 1.....	8.6	.....	20.45	.....	+0.0298
50 c.c. cyl. ord.....	.....	8.4	20.5	.....	-0.1702
50 c.c. cyl. cert.....	.....	8.6	20.3	.....	+0.0298
50 c.c. cyl. ord.....	.....	9.05	3.9	.....	+0.5098
10 c.c. cyl. cert.....	.....	8.7	4.00	.....	+0.1298
Lüttke-Martius .....	10.530	.....	.....	17.00	.....
Strauss tube 2.....	10.6	.....	16.89	.....	+0.07
50 c.c. cyl. ord.....	.....	10.7	16.6	.....	+0.17
50 c.c. cyl. cert.....	.....	10.65	16.7	.....	+0.12
10 c.c. cyl. ord.....	.....	11.05	3.2	.....	+0.52
10 c.c. cyl. cert.....	.....	10.8	3.3	.....	+0.27
Lüttke-Martius .....	11.349	.....	.....	15.6	.....
Strauss tube 2.....	Meniscus below scale	.....	.....	.....	.....
50 c.c. cyl. ord.....	.....	11.1	15.9	.....	-0.149
50 c.c. cyl. cert.....	.....	11.25	15.7	.....	-0.099
10 c.c. cyl. ord.....	.....	11.3	3.1	.....	-0.049
10 c.c. cyl. cert.....	.....	11.4	3.15	.....	+0.051

length and numerous graduations of its scale. With both kinds of cylinders, chlorid determinations can be made with accuracy equal to that of the Strauss tubes, and the advantages of their longer scales are shown



by the first and last analyses of the series in Table 5, in which the meniscus was once above and once below the limits of the Strauss scale. For chlorid estimations by this rapid method, the certified 50 c.c. cylinder is to be recommended especially. It has the advantage of being easily procured, of having accurate calibrations and a long, frequently divided scale, and in addition gives results that are considerably more accurate than those obtained by the Strauss tubes.

As the estimation of chlorids in the urine has diagnostic value in the study of renal function, as well as in the study of many diseases, an attempt was made to adapt the method of chloridometry in graduated cylinders to the requirements of urology. In studying renal function by means of collecting through a ureteral catheter the urine from one kidney, often only 1 c.c., or slightly more than this, is obtained. For the ordinary Strauss test this amount of urine is insufficient, and it is likewise not enough for the test when carried out in 50 c.c. cylinders. For this reason small 10 c.c. cylinders were employed in the series of analyses summarized in Table 5. One cylinder was an ordinary 10 c.c. graduated cylinder, the calibrations of which were inaccurate. The other was a somewhat longer 10 c.c. cylinder, graduated in 0.1 c.c., with accurate calibration. Into these cylinders 1 c.c. of urine and 2 c.c.  $N/10$   $AgNO_3$  (Lüttke-Martius silver solution) were placed. The titration was then done by adding  $N/20$  ammonium thiocyanate. In this way the relations between the solutions were kept the same as in the ordinary Strauss method. As one-fifth of all the solutions used in computing the scale for chloridometry in graduated cylinders, the results obtained with the 10 c.c. cylinders had only to be multiplied by 5 in order to allow reading from the amount of fluids in the cylinders the grams of chlorids per liter of urine tested, by means of the scale computed for the larger cylinders. Determinations made by these small cylinders, however, exhibited large discrepancies from the Lüttke-Martius figures. With the ordinary 10 c.c. cylinders errors ranged from 0.049 to 0.7189 gm. of chlorids per liter, while with the certified 10 c.c. cylinders they were from 0.016 to 0.27 gm. per liter. With the certified cylinder the readings were within the limits of desired clinical accuracy. But, in spite of this, these small cylinders cannot be recommended for use in making chlorid estimations. Great care must be exercised in putting the urine and silver nitrate solution in the cylinders, for when such small quantities are used, a drop of the solution left along the sides of the tube will have large effect on the result of the titration. It is advisable, therefore, when only small amounts of urine to be analyzed for chlorids are available, to dilute this urine carefully a sufficient number of times to give somewhat more than 5 c.c. More accurate estimations then can be made on the diluted urine by means of the Strauss method in the tubes or in the graduated cylinders than are possible with small cylinders, using small quantities of urine.

## THE METHOD OF ACHARD AND THOMAS

The other simple method for the determination of chlorids in the urine is that of Achard and Thomas.<sup>1</sup> These authors have devised an instrument comparable to the Esbach tube, and employ the process of Mohr<sup>4</sup> for the titration method. This method is based on the precipitation of chlorids by silver nitrate of known strength added in excess, and the titration for the excess of silver using potassium chromate as an indicator. In Mohr's procedure a certain amount of the urine is placed in a dish, to which is added, drop by drop, a standardized solution of silver nitrate (29.075 gm.  $\text{AgNO}_3$  dissolved in 1,000 c.c.  $\text{H}_2\text{O}$ ; of this solution, 1 c.c. will precipitate 0.01 gm.  $\text{NaCl}$ ). To denote when all the chlorids of the urine have been precipitated a drop or two of a solution



Fig. 2.—Chloridometer of Achard and Thomas.

of potassium chromate are added as an indicator. When the precipitation of chlorids is complete, a reaction takes place between the silver and the chromate, forming silver chromate, which gives a brownish-red color to the mixture. The appearance of the color indicates the end of the reaction, and at this point the amount of chlorids present in the urine is determined from the quantity of the silver used.

The approximate estimation of the chlorids which Achard and Thomas have introduced, rests on the same reaction, but proceeds in the opposite direction. Instead of adding to a fixed amount of urine a variable quantity of silver nitrate, the quantity of urine is varied, while that of the silver nitrate remains constant. This conveniently permits employing only a small amount of the standardized solution of silver

nitrate, and on this basis the method is recommended by the authors as a bedside procedure. The potassium chromate is added to the silver nitrate in the tube before the addition of any urine. Silver chromate is formed at once, giving the mixture a deep red color. To this, the urine is added and the tube inverted several times. The chromate of silver is decomposed by the successive addition of urine and silver chlorid and a yellowish precipitate is formed. The end of the reaction occurs when all of the reddish-brown color disappears, and gives place to a light yellow color throughout the fluid and precipitated contents of the tube. For this test the urine should be slightly acid, rendered so, if necessary, by the addition of a little acetic acid.

The instrument<sup>15</sup> shown in Figure 2, employed by Achard and Thomas, is a tube not unlike the Strauss chloridometer. This tube is closed by a rubber stopper; its barrel bears a special calibration to indicate the amount of chlorids in the urine thus tested. At the bottom, a first graduation, marked A, indicates the fixed quantity of the standard solution of silver nitrate to be placed in the tube. The quantity is 5 c.c. Above this mark, the graduations of the scale indicate in grams per liter various amounts of chlorids.

The scale is constructed according to the following table:

3.3 c.c. correspond to	15.0 gm. chlorids per 1,000 c.c. of urine.
5.0 c.c. correspond to	10.0 gm. chlorids per 1,000 c.c. of urine.
7.1 c.c. correspond to	7.0 gm. chlorids per 1,000 c.c. of urine.
10.0 c.c. correspond to	5.0 gm. chlorids per 1,000 c.c. of urine.
12.5 c.c. correspond to	4.0 gm. chlorids per 1,000 c.c. of urine.
14.0 c.c. correspond to	3.5 gm. chlorids per 1,000 c.c. of urine.
16.0 c.c. correspond to	3.0 gm. chlorids per 1,000 c.c. of urine.
20.0 c.c. correspond to	2.5 gm. chlorids per 1,000 c.c. of urine.

The total volume in the tube at any of these degrees is the amount given here plus the 5 c.c. of silver nitrate solution.

To make determinations with this tube the following procedures must be carried out:

The tube is filled to the mark A with the standardized solution of silver nitrate (29.075 gm.  $\text{AgNO}_3$  to 1,000 c.c.  $\text{H}_2\text{O}$ ). To this 3 or 4 drops of a 1:5 solution of potassium chromate are added. The undiluted urine is then added gradually, mixing the fluids constantly by gentle inversion of the tube. When the reddish-brown color of the mixture finally changes to a permanent yellow, the end-point is reached. The chlorids present in grams per liter are read off directly from the scale.

The authors advise using the upper limit of the scale because of the greater accuracy of readings in these regions. If the amount of chlorids in the urine is above 5 gm. per liter on the first test, a second determination should be made with diluted urine. Above 5 gm. of chlorid per

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15. The Achard and Thomas chloridometer is manufactured by Paul Altmann of Berlin. Price 3 marks.

liter make dilutions of 1 to 2, above 10, a dilution of 1 to 4, above 15, dilute 1 to 6. The results thus obtained are to be multiplied by the amount of the dilution to estimate the quantity of chlorids in the undiluted urine. When the chlorids are less than 2.5 gm. per liter, the silver nitrate must be diluted; when, to calculate the true chlorid content of the urine, the observed content should be divided by the degree of the dilution.

TABLE 6.—ESTIMATION OF CHLORIDS IN AQUEOUS SOLUTIONS BY ACHARD-THOMAS METHOD COMPARED WITH LÜTTKE-MARTIUS METHOD

Lüttke-Martius Method	Grams NaCl per liter by					
	Achard-Thomas					
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
0.5	0.483	0.5	0.583	.....	.....	.....
1.228	1.03	1.0	1.0	1.0	1.0	1.03
1.638	1.33	1.25	1.666	1.5	1.33	1.33
2.106	1.91	1.91	1.8	2.16	2.	1.91
2.574	2.7	2.8	2.75	2.8	2.75	2.8
3.276	3.2	3.25	3.2	3.25	3.2	3.2
3.627	3.6	3.5	3.7	3.55	3.55	3.55
3.9953	4.1	4.05	4.05	4.1	4.1	4.0
4.797	4.5	4.7	4.7	4.7	4.5	4.5
5.085	5.2	5.1	5.2	5.1	5.05	5.0
5.5867	5.35	5.7	5.8	5.9	5.5	5.9
6.101	6.0	6.0	6.1	6.1	6.0	6.0
6.5227	6.5	6.5	6.7	6.5	6.5	6.4
7.254	7.05	7.0	7.1	6.9	7.0	6.9
7.605	7.6	7.5	7.4	7.5	7.2	7.1
8.1607	8.0	7.5	8.0	8.0	7.9	8.0
8.5702	8.0	8.4	8.4	8.3	8.3	8.5
9.0967	9.0	8.5	9.0	8.5	8.5	9.0
9.5355	9.2	9.5	9.5	9.0	9.5	9.5
10.2667	10.1	10.0	10.1	10.1	10.1	10.0
10.764	10.5	10.1	10.5	10.5	10.2	10.2
11.0565	12.0	11.0	11.0	11.0	11.0	11.0
Max. difference from Lüttke-Martius	0.9435	0.664	0.264	0.5967	0.5967	0.564
Min. difference	0.017	0.0	0.028	0.0115	0.0385	0.076
Average difference	0.4802	0.332	0.146	0.304	0.3175	0.320

This instrument and method were tested as follows: (a) Tests of the calibration; (b) estimation of chlorids in aqueous solution; (c) estimation of chlorids in normal urine, undiluted; (d) estimation of chlorids in undiluted urine containing albumin. In all titrations results obtained by the Lüttke-Martius method were taken as the absolute for comparison.

(a) *Tests of the Calibration.*—In comparing by buret readings the calibration of six of these tubes with the table given by Achard and



Thomas, it was found that the difference between the actual and required cubic content of the tube between the marks of the scale was never more than 0.2 c.c. On the whole, the scale marks were found to be accurately placed.

(b) *Estimation of Chlorids in Aqueous Solution.*—The results of determinations of chlorids in aqueous solution by the Achard-Thomas method as compared with the Lüttke-Martius method are shown in Table 6. The Achard-Thomas determinations are seen to vary from the Lüttke-Martius by as much as 0.9435 gm. of chlorids per liter. Readings were obtained between this degree of error and no error. Nearly all readings were too high. The average error of all readings was about 0.3 gm. per liter. As this series of solutions was the same as that used in testing the Strauss tubes, comparison between these results may be made by reference to Table 2. From this it will be seen that the error in the Strauss determinations (0.2 gm. per liter) were considerably less than this, and in no instance were even one-half as great as some of the errors in the Achard and Thomas estimation.

The explanation of these large errors is provided by the character of the chemical reaction and the construction of the tubes. It has long been admitted that the Mohr method for the estimation of chlorids gives too high results, and this may be taken as one factor in the error under consideration. Furthermore, the end-point of the titration reaction is not a sharp one, especially under the conditions of the analysis in the Achard-Thomas tube. The final color of the titration in the tube does not come suddenly, as in the Strauss method, but develops gradually. A good yellow color is obtained in the fluid of the tube long before all of the brownish-red precipitate disappears. Scale readings of varied degrees, therefore, may be made according as the end-point is judged by the permanent yellow color in the fluid (as the directions specify), or by the complete change in the precipitate (as the chemistry of the reaction demands). This introduces another source of error. All my readings were taken when the precipitate changed completely from brown to yellowish white, as I found that this point gave the most accurate results as compared with the Lüttke-Martius figures. In addition, the scale itself is a source of error. It is constructed with relatively few marks, separated by considerable and varying distances on the tube, with no register between them. The readings, few of which corresponded exactly to the marks on the scale, were taken as the nearest approximations that judgment allowed.

(c) *Estimation of Chlorids in Normal Urines.*—Table 7 presents the results obtained by chloridometry performed on the same series of normal urines used for Table 3, for the Strauss tubes. These results are more like the Lüttke-Martius figures than those in the above table when

aqueous solutions of sodium chlorid were used. The errors in some cases are considerable, however, being as high as 1.299 gm. per liter. This is much a larger error than any in the Strauss determinations, and the use of the tubes in these tests exhibit the same difficulties of inconvenient scale, approximate readings, and uncertain end-point as were described above. Their advantage over the Strauss method is that the color of the urine makes no difference at all in the distinctness of the end-color, when finally this is obtained. Estimations by this method can be made somewhat more rapidly than by the Strauss process.

TABLE 7.—ACHARD AND THOMAS TUBES WITH CHLORIDS OF NORMAL URINE

Method	Gm. NaCl Per Liter					
Lüttke-Martius .....	2.0475	4.9725	6.903	7.722	9.716	11.349
Achard-Thomas —						
Tube 1 .....	2.0	.....	7.0	8.2	8.5	10.05
Tube 2 .....	2.0	4.8	6.8	7.5	9.0	11.0
Tube 3 .....	.....	5.0	6.9	8.0	9.0	10.05
Tube 4 .....	.....	.....	7.0	7.6	8.0	.....
Tube 5 .....	.....	.....	.....	.....	9.0	.....
Tube 6 .....	.....	.....	.....	.....	9.0	.....
Max. difference .....	0.0475	0.1725	0.103	0.478	1.216	1.299
Min. difference .....	0.0475	0.0275	0.003	0.122	0.716	0.349
Mean. difference .....	0.0475	0.10	0.052	0.300	0.866	0.824

(d) *Estimation of Chlorids in Albuminous Urine.*—A series of urines containing the proteins of blood-serum were analyzed for their chlorid content by the Achard-Thomas method. This series was the same as that for the Strauss tubes, shown in Table 4. Chlorid determinations were made on urines to which salt-free protein had been added in quantities up to 27 gm. per liter. The titrations were all within the limits previously discovered to represent the accuracy of these tubes. In no case was the presence of protein found to interfere with the Achard-Thomas process.

Tests of this method show that because of the inconveniences of its irregular scale, and the uncertainty of its end-point, the Achard-Thomas process is capable of giving only widely approximate results. It has the advantage of being rapid and uninfluenced by the color of the urine tested, while it requires only one standardized solution. But its inaccuracies are so great that its value as a clinical method is considerably less than that of the process of Strauss.

#### CHLORIDOMETRY BY ACHARD-THOMAS METHOD IN GRADUATED CYLINDERS

This method, like that of Strauss, may be simplified further by eliminating the special apparatus, the chloridometer tube, and employing a graduated 50 c.c. cylinder. The titration may be made in one of these

cylinders and the total volume of fluid noted at the end of the analysis. From the amounts of standard silver nitrate solution, the quantity of urine added and their chlorid equivalent, a formula may be derived by which the total volume of fluid at the end of the titration may be converted into grams of chlorid per liter of urine. Into the cylinder 5 c.c. of standardized Achard and Thomas silver nitrate solution are placed. Of this solution, 1 c.c. will precipitate 0.01 gm. NaCl. To this are added 2 to 3-drops of potassium chromate. Urine is then added until the yellow color appears, determining the end-point as in the ordinary manner. From these quantities the following formula is derived:

$V$  = total volume of fluid in cylinder at end of titration ( $V - 5$ ) = amount of urine added.

$5 \times 0.01 = 0.05$  = amount of NaCl precipitable by 5 c.c. of  $\text{AgNO}_3$  solution.

As the chlorid content of the amount of urine added ( $V - 5$ ) is 0.05 gm. when the end-point is reached, then

$$\begin{aligned} \text{Grams NaCl per c.c.} &= \frac{0.05}{V - 5} \\ \text{Grams NaCl per c.c.} \times 1,000 &= \text{grams NaCl per liter.} \\ \therefore \text{Grams NaCl per liter} &= \frac{0.05 \times 1,000}{V - 5} = \frac{50}{V - 5} \end{aligned}$$

Example: Suppose  $V = 19$

$$\text{Gm. NaCl per liter} = \frac{50}{19 - 5} = \frac{50}{14} = 3.57.$$

This result corresponds to the figures in the table given by Achard and Thomas.

In applying this method, as described above, or by reversing the procedure, adding the silver nitrate solution to the urine, as in the Mohr technic, unsatisfactory results were obtained. The short scale length and the uncertainty of the end-point make the results of titrations in graduated cylinders quite inaccurate. The readings obtained by the cylinders, however, were as accurate as those by the tubes of Achard and Thomas, and the cylinders have the advantage of providing a scale of more calibrations than that on the tubes. But the errors are so great that they prevent recommendation of the method for clinical use.

#### CONCLUSIONS

Several simplified methods for estimating chlorids in the urine are described and tested. Of these procedures:

1. The Strauss method is found to be simple and rapid and to give results, which, while not exact enough to serve as a basis for metabolic experiments, are sufficiently accurate for clinical purposes.

2. The Strauss method is directly applicable to both normal and albuminous urine.

3. The Strauss method modified by performing the test in a graduated 50 c.c. cylinder, instead of the Strauss tube, gives results more accurate than the original method, and is a simple R process. A scale is computed for use with such cylinders.

4. The Achard and Thomas method is simple and rapid, but because of uncertain end-point and inconvenient scale, is not sufficiently accurate.

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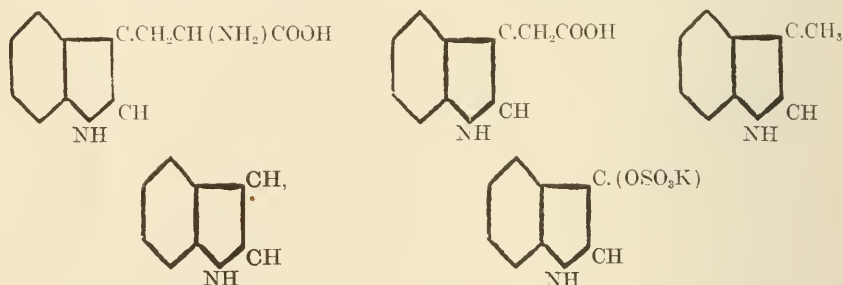


# A PRELIMINARY NOTE ON THE EXCRETION OF INDOL-ACETIC ACID IN THE URINE\*

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In making metabolism studies in cases of dementia praecox I was struck by the appearance of a red color in the urines which had been treated with strong mineral acids. The red substance was found to be insoluble in ether, chloroform, benzene, toluene and xylene, but soluble in water, ethyl alcohol and amyl alcohol. In amyl alcohol the red substance gave an absorption spectral band between 556 and 570. This compound with these characteristics seems to be identical with the uroosein described by Nencki and Sieber,<sup>1</sup> H. Rosin,<sup>2</sup> C. A. Herter,<sup>3</sup> J. Ph. Staal,<sup>4</sup> L. C. Willard,<sup>5</sup> V. Arnold<sup>6</sup> and A. Ellinger and A. Flammmond.<sup>7</sup> According to the work of Herter, the uroosein is formed from indol-acetic acid by the action of a strong mineral acid and an oxidizing agent.

That indol-acetic acid is derived from tryptophan can hardly be doubted, for first the indol group is common to both, and second, tryptophan is abundant in animal tissues and in the daily diet of man. The close chemical relationship between tryptophan, indol-acetic acid, skatol, indol and indican is clearly shown by the following graphic formulas:



Skatol and indol are well known toxic substances, which, in normal metabolism are made non-toxic by their transformation into ethereal sulphates, one of which is indican.

\* From the Laboratory of the Illinois State Psychopathic Institute.

\* Submitted for publication June 11, 1913.

1. Jour. f. prakt. Chemie, 1882, xxvi, 333.

2. Deutsch. med. Wehnschr., 1893, p. 51.

3. Jour. Biol. Chem., 1908, iv, 239.

4. Ztschr. f. physiol. Chemie, 1905, xlv, 236.

5. Ztschr. f. physiol. Chemie, 1905, xlv, 515.

6. Ztschr. f. physiol. Chemie, 1909, lxi, 240.

7. Ztschr. f. physiol. Chemie, 1909, lxii, 276.

Nencki and Sieber<sup>1</sup> were not able to find indol-acetic acid in the urines of normal persons. Rosin<sup>2</sup> claimed to have found it in every normal urine examined, and in greatest amount when the diet consisted of vegetables. Garrod and Hopkins<sup>3</sup> say that it occurs only occasionally in normal urines. Herter claims that with potassium nitrite and concentrated hydrochloric acid it was impossible to obtain the reaction except in very faint suggestions from any normal urine examined. Nencki and Sieber report the presence of "urorosein" in the urines of patients suffering with osteomalacia, nephritis, typhoid fever, carcinoma of the esophagus, ulcer of the stomach and perityphlitis. They say that 10 per cent. of all diseased persons gave a positive reaction. Herter reported indol-acetic acid in the urine of an under-developed child suffering from a peculiar intestinal putrefaction.

Since the findings have been somewhat contradictory thus far, it was thought best to examine the urines of a number of apparently healthy persons. The urines of ninety-one employees of the Kankakee State Hospital were collected and tested for indol-acetic acid. The individuals were all young, working and apparently in good health. The following tests were made on each urine: Ten c.c. of urine in a test-tube was treated with about 3 c.c. of hydrochloric acid (sp. gr. 1.19) and 2 drops of 0.2 per cent. sodium nitrite. The contents of the tube were mixed and allowed to stand ten minutes. If a pink or red color appeared the test for indol-acetic acid was called positive. The second test was identical with the first, except that no nitrite was used.

TABLE 1.—TESTS ON URINES OF YOUNG, HEALTHY PERSONS

	No. of Cases	Test without O-Agent		Test with O-Agent	
		Number Positive	Per Cent. Positive	Number Positive	Per Cent. Positive
Men .....	68	5	7.35	13	19.12
Women ....	23	2	8.70	6	26.09
Total .....	91	7	7.69	19	21.37

These results indicate that indol-acetic acid may appear in the urine of apparently healthy young people.

As I first noticed indol-acetic acid in the urine of insane patients in quantities relatively very large, an examination was made of a large number of such urines. The results of the tests similar to those described above were as shown in Table 2.

S. Jour. Physiol., xx, 112.

TABLE 2.—TESTS OF URINES OF INSANE PATIENTS

Type of Mental Disorder	No. of Cases	Test without O-Agent		Test with O-Agent	
		No. Positive	Per Cent. Positive	No. Positive	Per Cent. Positive
Organic brain disease (not differentiated) . . . . .	7	1	14.29	2	28.58
General paralysis of the Insane . . . . .	21	2	9.52	4	19.04
Senile dementias . . . . .	16	2	12.50	10	62.50
Infective exhaustive psychoses . . . . .	3	1	33.30	3	100.00
Intoxication psychoses (alcohol and morphin) . . . . .					
Chronic . . . . .	14	1	7.14	4	28.57
Acute . . . . .	6	1	16.67	2	33.33
Total . . . . .	20	2	10.00	6	30.00
Dementia praecox group					
Hebephrenic . . . . .	42	4	9.52	15	35.71
Katatonic . . . . .	12	5	41.66	10	83.33
Paranoid . . . . .	18	2	11.11	10	55.55
Not differentiated . . . . .	102	8	7.84	48	47.06
Total . . . . .	174	19	10.92	83	47.70
Manic depressive group					
Depressed . . . . .	16	1	6.25	6	37.50
Excited . . . . .	17	2	11.76	4	23.52
Remission . . . . .	2	0	....	0	....
Total . . . . .	35	3	8.57	10	28.57
Involucional melancholias . . . . .	5	0	....	2	40.00
Psychoneuroses . . . . .	2	0	....	2	100.00
Paranoic states . . . . .	4	1	25.00	3	75.00
Psychopathic personalities . . . . .	3	0	....	1	33.33
Epileptic psychoses . . . . .	42	3	7.14	15	35.71
Defective mental development . . . . .	26	3	11.54	15	57.70
Unclassified cases of insanity* . . . . .	132	18	13.64	65	49.24
Tuberculosis in insane individuals† . . . . .	26	3	11.54	10	38.46
Very inactive insane individuals . . . . .	25	4	16.00	13	52.00
Total insane individuals tested . . . . .	490	55	11.22	211	43.06

\* Old cases which are largely cases of dementia praecox undoubtedly.

† These individuals were also included in the different psychosis groups.

Of the 490 urines from insane patients, 43.06 per cent. contained indol-acetic acid as compared with 21.37 per cent. in the case of normal urines. Such a large difference indicates a relation between indol-acetic acid excretion and some condition frequent among the insane. The data in Table 3 exclude the validity of the claim that the age factor is an influential one.

In many cases the number of patients of a certain type of psychosis is too small for the percentages of positives to be of any considerable value alone. It will, however, be noted that where the psychoses seem to arise on a basis of constitutional inferiority, the percentage of positive reactions is generally relatively high. The best example of this is found in the case of the dementia praecox group with 174 cases and 47.7 per cent. positive, and in the case of the mental defectives with twenty-six cases and 57.7 per cent. On the other hand, the best examples of low percentages are found in the group of general paralysis with twenty-one cases and 19.04 per cent. positive, and the intoxication group with twenty cases and 30.00 per cent. positive. The intoxication psychoses and general paralysis of the insane may both be considered as more definitely acquired conditions, since they require the presence of some exogenous

TABLE 3.—AGE FACTOR IN INDOL-ACETIC ACID EXCRETION

	No. of Cases	Test without O-Agent		Test with O-Agent	
		No. Positive	Per Cent. Positive	No. Positive	Per Cent. Positive
Insane individuals of ages between—					
15-25 .....	32	.....	.....	14	43.75
25-40 .....	165	.....	.....	76	46.06
40-60 .....	203	.....	.....	93	45.85
60—above .....	57	.....	.....	23	40.35

factor. Dementia praecox and defective mental development, on the other hand, are generally believed to be more definitely constitutional in nature. This apparent relation between constitutional inferiority and indol-acetic acid excretion is strengthened when we compare the percentage of positive among the cases belonging to the dementia praecox group with the percentage of positives among the insane other than dementia praecox. This point is clearly shown in Table 4.

There are at least two possibilities to be considered in connection with the occurrence of indol-acetic acid in the urine. First, like homogentisic acid and cystin, it may indicate a constitutional disorder of metabolism. The figures given above tend to suggest that this is true, although it cannot yet be considered as proven. Secondly, it may result from a particular kind of intestinal putrefaction, for Hopkins and Cole<sup>9</sup> have

9. Jour. of Physiol., 1903, xxix, 29-451.



shown that indol-acetic acid can be produced *in vitro* from tryptophan by bacterial growth. Work along these lines is being actively carried on at the present time in this laboratory.

TABLE 4.—COMPARISON OF POSITIVE CASES AMONG CASES OF DEMENTIA PRAECOX AND OTHER FORMS OF INSANITY

	No. of Cases	No. Positive	Per Cent. Positive	No. Positive	Per Cent.
Sane persons apparently healthy .....	91	7	7.7	19	21.4
Insane persons, exclusive of dementia praecox and unclassified groups .....	184	18	9.8	63	34.2
Dementia praecox group....	174	19	10.9	83	47.7

Another point also needs further study. It will be noted that to some urines an oxidizing agent had to be added in order to convert the indol-acetic acid to uroscopine, whereas in others no such addition was necessary. Herter commented on this fact and showed that a positive uroscopine reaction might appear without adding an oxidizing agent if the urine containing indol-acetic acid were allowed to stand twelve to twenty-four hours, and claims to have proven that this was due to the growth of nitrifying organisms whereby the necessary nitrite was provided. This certainly is not necessary in all cases, for a positive reaction without the addition of any oxidizing agent has been obtained in specimens immediately after they are voided. It therefore remains to explain the presence of an oxidizing agent in some urines. This point is also being investigated in this laboratory.

The author is indebted to Dr. H. Douglas Singer for much aid in collecting material and many valuable suggestions in carrying out the work.

# The Archives of Internal Medicine

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Vol. XII

AUGUST 1913

No. 2

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## A STUDY OF THE VENTRICULAR SYSTOLE—SUBCLAVIAN INTERVAL, WITH A DISCUSSION OF THE PRESPHYGMIC PERIOD \*

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### INTRODUCTORY

The material on which this paper is based was obtained at the suggestion of Prof. Friedrich von Müller, in his wards at the *Allgemeine Krankenhaus, links der Isar*, in Munich. We intended originally to take as the subject of the paper, the presphygmic period, i. e., the time between the beginning of ventricular systole and the opening of the aortic valves. As our work advanced, however, we had reason to doubt the accuracy of the presphygmic period as determined by our method, or, indeed, by any present method (except possibly in the case of aneurysms), and we, therefore, decided to discuss primarily the  $V_s$ -S time, or the time passing between the beginning of ventricular systole, and the arrival of the pulse wave at a given point on the subclavian artery.

As we shall show, the presphygmic period obtained in the usual way varies directly as the  $V_s$ -S time, and, indeed, is nothing but the  $V_s$ -S time with a fairly constant fraction of time subtracted. The significance of the two is, therefore, the same, and, in spite of the doubtful accuracy of the presphygmic period, we have in every case determined it, and have tabulated it, together with the  $V_s$ -S time.

### METHOD

The tracings were all made with the Jacquet sphygmograph, running at high speed, i. e., 1 cm. = 1/5 second. Unless otherwise stated, the patients were all in the recumbent position, and usually turned slightly toward the left side, in order to obtain the best possible cardiogram. Simultaneous tracings were made of the apex beat, subclavian or carotid, and radial pulses. When possible, we used the subclavian in preference to the carotid pulse. The site selected was either on the subclavian artery

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\* From the Second Medical Clinic, Munich. Prof. Friedrich v. Müller, director.

\* Submitted for publication April 9, 1913.

above the clavicle 10 to 11 cm. from the second costosternal junction, or on the carotid an average distance of 15 cm. from the same point.

When satisfactory tracings had been obtained, foot-points of required waves were marked off on the time-line with a pair of calipers and measured, and, to correct any error due to slight variations of speed, each 1/5 second division of the time line involved was also measured, and in this way exact times computed. In order to insure an accurate average, a number of measurements of each required interval was made in every

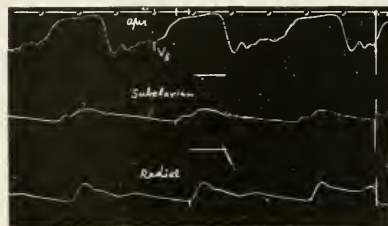


Fig. 1.—Case 15. Shows a cardiogram in which a jugular tracing (Tracing 2) helps to identify the correct foot-point.

instance, except in arrhythmias, where this is obviously impossible, and, unless the greatest variation was so slight as to fall within the limits of technical error, the tracings were discarded. The limit of technical error we called .01 second.

It is very hard in the majority of cases to obtain tracings in which the cardiogram and two pulses are all sufficiently definite and have sharp

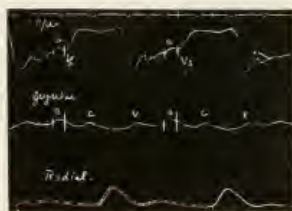


Fig. 2.—Case 15. The "a" wave in the jugular tracing identifies the point  $V_s$  as the correct beginning of the upstroke in the cardiogram. That this point coincides with  $V_s$  in Tracing 1 is proved by the fact that the  $V_s$ —R times are the same in both tracings.

enough foot-points to use for purposes of measurement. The chief difficulty is perhaps encountered, as others<sup>1</sup> have pointed out, in getting a cardiogram in which there is a sharp foot-point, and about which there is no doubt as to where the true upstroke begins. This is particularly

1. Robinson, G. C., and Draper, G.: A study of the Presphygmie Period of the Heart. *THE ARCHIVES INT. MED.*, 1910, v, 169.

hard and, in fact, usually impossible when the "auricular flip" is either very large, or absent. Occasionally simultaneous jugular tracings may settle the question (see Tracings 1 and 2), but as a rule, such cardiograms must be excluded.

### *The Ventricular Systole-Subclavian ( $V_s-S$ ) and Ventricular Systole-Carotid ( $V_s-C$ ) Intervals*

The  $V_s-S$  time is obtained by making a simultaneous tracing of the apex beat and subclavian pulse, and calculating the time between the two foot-points in the manner described above. The  $V_s-C$  time is obtained in exactly the same way, except that the carotid pulse is used in place of the subclavian. The difference between the two is very small — the  $V_s-S$  time averaging .005 to .006 second less than the  $V_s-C$  time — which is of no practical importance, lying, as it does, within the limits of technical error. We found the  $V_s-S$  time the more satisfactory and have used it throughout, unless otherwise stated.

The  $V_s-S$  time represents the presphygmie period plus the time taken by the pulse to travel from the aortic valves to the receiver on the subclavian artery. Variations in length may, therefore, represent changes in the presphygmie period or pulse speed, or both. It has commonly been assumed that the presphygmie period was the main factor, and that the changes due to pulse speed could be ignored. Keyt<sup>2</sup> held that the  $V_s-C$  time and presphygmie period ran parallel, variations of one corresponding accurately to variations of the other. It seems, indeed, unlikely, as Keyt suggested, that the pulse in traveling 15 cm., which should take only about .021 second, could vary in speed sufficiently to cause any significant change in the  $V_s-C$  time.

### *The Relation of the Presphygmie Period to the $V_s-S$ Time*

Previous observers<sup>3</sup> have ranged the normal presphygmie period vari-  
ously from .02 to .154 second. The methods most commonly used have been: (a) Measurement of the ascending limb of the cardiogram from

2. Keyt: Sphygmography and Cardiography. New York and London, 1887.  
3. For references see Robinson and Draper. (Note 1.)

Observer	P. P. in Seconds	Observer	P. P. in Seconds
Jacquet and Metzner.....	.02 —.03	Rive .....	.073
Schmidt .....	.02 —.04	Hilbert .....	.06 —.12
Müller .....	.025—.07	Hoeckhaus .....	.07 —.10
Reek .....	.045—.06	Martius .....	.07 —.14
Tigstedt .....	.051	Luciani .....	.08 —.10
Keyt .....	.06	Von Ziemssen and Maxi- movitch .....	.08 —.17
Hürthle .....	.06	Landois .....	.085
Einthoven and Geluk ....	.06	Edgren .....	.087—.093
Grunmach .....	.07	Erlanger .....	.12 —.18
Van Jürgen .....	.07	Robinson and Draper...	.07 —.085



the foot-point to notch P; (b) calculation from tracings of the pulse and the exposed heart; (c) measurement of the E-wave of aortic aneurysm; (d) obtaining the time between the foot-point of the cardiogram and the pulsation of aneurysm of the ascending aorta (probably the most accurate method); (e) calculation from tracings of the apex beat and the carotid or subclavian pulse, with or without correction for the time of pulse transmission. The last, with correction, which is the method we have used, is the one adopted by most recent observers, and is probably, with the exception of the aneurysm method, the most accurate.

Simultaneous tracings are made of the apex beat, subclavian or carotid, and radial pulses. The pulse speed is then obtained from the S-R (subclavian-radial) or C-R (carotid-radial) time, and from this the A-S time (pulse time from the aortic valves to the receiver on the subclavian) is computed. This subtracted from the  $V_s$ -S time gives the presphygmie period. The fallacy of this method lies in the assumption that the pulse speed is the same in the arm as in the thorax. Though Nicolai<sup>4</sup> thought that the pulse in the aorta and the carotid artery should be faster than in the arm, others<sup>5</sup> have shown quite conclusively that it is slower, and have suggested that in getting the presphygmie period too little had been subtracted from the  $V_s$ -C time. That not enough has been subtracted there can be little doubt, and we have some reason to believe, from work subsequently done by one of us (Swann), that a much greater delay may occur in the aorta than has heretofore been suspected, and that the presphygmie period, instead of being normally 0.07 to 0.085 second, as we have determined it to be by our present method, may be more nearly 0.055 to 0.07 second, which corresponds closely with v. Müller,<sup>6</sup> Reck,<sup>7</sup> Tigerstedt,<sup>8</sup> Keyt,<sup>2</sup> Hürthle<sup>9</sup> and Einthoven and Geluk.<sup>10</sup> Until some means is devised for recording the opening of the aortic valve, the present method will probably remain the most accurate at our disposal, and we have, therefore, in every case, calculated the presphygmie period in this way and tabulated it for comparison with the results of other observers.

4. Nicolai: *Handbuch der Physiologie des Menschen*, Brunswick, 1909, i, 787.

5. Keyt: (See Note 2) especially. Also Grunmach,<sup>4</sup> Robinson and Draper (Note 1), and Grunmach: *Ueber die Pulsgeschwindigkeit bei Erkrankungen des Circulations-Apparates sowie bei Einwirkung toxischer Mittel*. Virchows Arch. f. path. Anat., 1885, cii, 565.

6. v. Müller, F.: *Einige Beobachtungen aus dem Percussionseurs*. Berl. klin. Wchnschr., 1895, xxxvi, 783.

7. Reck: *Graphische Untersuchungen über normale und pathologische Herzstossformen*. Inaug. Diss. Bonn., 1890.

8. Tigerstedt: *Die Puls-Kurve der Aorta beim Menschen*. Skand. Arch. f. Physiol., 1908, xx, 249; *Ergebn. der Physiol.-Weisbaden*, 1902, i, Part 2, 253.

9. Hürthle: *Beiträge zur Hämodynamik*. Arch. f. d. ges. Physiol. (Pflüger's), 1891, xlix, 29.

10. Einthoven and Geluk: *Die Registrirung der Herztöne*. Arch. f. d. ges. Physiol. (Pflüger's) 1894, lvii, 617.

*The Subclavian-Radial (S-R) Time and Pulse Speed*

After a careful investigation of the subject, Keyt<sup>12</sup> concluded that in the same individual the pulse speed in a given vessel varied but little with changes of pulse rate and other changes of the circulation, though varying greatly in different individuals, and somewhat in different vessels of the same individual. Our own observations<sup>11</sup> and those of others<sup>12</sup> have confirmed this.

For computing the pulse speed we have used, when possible, the subclavian and radial pulses, the distance between the two points used averaging 55 to 56 cm. The following tabulation shows the average pulse speed for all our cases with regular heart action:

Average speed — 7.09 metres per second, or 1 metre in .141 second.  
 Slowest speed — 4.52 metres per second, or 1 metre in .221 second.  
 Fastest speed — 10.31 metres per second, or 1 metre in .091 second.

Average S-R time — .082 second.  
 Longest S-R time — .126 second.  
 Shortest S-R time — .056 second.

Average A-S time (by calculation) — .015 second.\*  
 Longest A-S time (by calculation) — .0219 second.  
 Shortest A-S time (by calculation) — .0114 second.  
 Average A-C\* time (by calculation) — .021 second.

It is evident from the above figures that if we subtracted 0.15 second from the A-S time in every case, we should have practically the same result as we obtain by the present method of determining the presphygmie period. For, if we substitute .015 second for the A-S time, as calculated in the two given cases, which represent the slowest and the fastest pulse speed obtained by us, we have errors of only .0036 second and .0069 second, respectively, which fall well within the limits of technical error.

Other observers have given the pulse speed as follows:

8.	metres per second.	Keyt. <sup>12</sup> Carotid-radial.
6.8	metres per second.	Erlanger. <sup>13</sup> Brachial and radial arteries.
6.15	metres per second.	Grummach. <sup>14</sup> Heart to radial.
6.	metres per second.	Hürthle. <sup>15</sup>
9.42	metres per second.	Landois. <sup>14</sup> Arm arteries.
5.33	metres per second.	Jacquet and Metzner. <sup>15</sup> Innominate and carotid arteries.
8.33 — 20	metres per second.	Robinson and Draper. <sup>1</sup>

11. Two exceptions are Cases 17 and 21, in which the S-R time varied distinctly with changes of posture.

12. Garrod, A. H.: Some Points Connected with the Circulation of the Blood Arrived at from a Study of the Sphygmograph Trace. Proc. Roy. Soc. London, 1874, xxiii, 140; also Robinson and Draper, Note 1.

13. Erlanger: Cardiograms Obtained from a Case of Operative Defect in the Chest Wall. Bull. Johns Hopkins Hosp., 1905, xvi, 394.

14. Landois: Die Lehre vom Arterienpuls. Berlin, 1872, p. 304.

15. Jacquet and Metzner: Cardiographische Untersuchungen an einem Falle von Fissura Sterni. Deutsch. Arch. f. klin. Med., 1901, lxx, 57.

16. Keyt (Note 2) made the average A-C\* time .026 second, seven inches from the second interspace. This would correspond to .022 second, 15 cm. from this point, coinciding closely with our own figures.

A glance at the above figures and at Table 1 shows how widely the speed has varied in our different cases. It shows, moreover, a conspicuous lack of conformity to any rules. It bears no constant relation to the  $V_s$ -S time,<sup>17</sup> nor does it vary consistently with the strength of systole, with the pulse rate, nor with different valvular lesions. In fact, the only rule which does seem to exist is that high blood-pressure produces a high speed, and low blood-pressure a low one. Thus Case 22, with the slowest speed, has a blood-pressure of 95/75, while Case 17, with the greatest speed, has a pressure of 190/170. This occurs in the majority of cases, although there are occasional exceptions.

#### RESULTS OF PRESENT INVESTIGATION

##### *The Normal $V_s$ -S Time*

As the  $V_s$ -S time varies somewhat in different individuals, it is difficult to set the normal limits very definitely. From results obtained in normal cases, and in cases in which, though not entirely normal there seemed to be no factors which should affect the  $V_s$ -S time, we have placed the normal time at from .085 to .10 second. Our normal presphygmie period would then be .015 second less, or .07 to .085 second, which corresponds exactly with the presphygmie period, as set by Robinson and Draper.<sup>1</sup>

##### *The Relation of the $V_s$ -S Time to Heart Muscle Efficiency*

Certain abnormal conditions of the circulation produce marked and fairly constant variations in the length of the  $V_s$ -S time. Thus, inefficient heart muscle and certain valvular lesions tend to lengthen it, while arteriosclerosis and other valvular lesions tend to shorten it. It is markedly affected by changes in posture, and somewhat by extremes of pulse pressure. Two or more of these conditions may occur together, in which case the effect of one may be increased or offset by the effect of others.

The question of the  $V_s$ -S time in its relation to heart muscle efficiency is interesting, and may be of value in diagnosis and prognosis, as has been suggested by former observers.<sup>1</sup> As they have pointed out, however, arteriosclerosis and other disturbing factors which may coexist, often make it impossible to determine just how much influence any one factor exerts, the  $V_s$ -S time therefore, being of definite clinical value only in cases in which there is an absence of disturbing factors. There can be little doubt that the  $V_s$ -S time is lengthened whenever the heart muscle is unable to meet the demands put on it, and it is probable that an

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17. Robinson and Draper (Note 1) found that in their cases it bore no relation to the presphygmie period.

abnormally long  $V_s$ -S time, if this time were always measured, would often be the first obtainable evidence of impending cardiac failure. Of interest in this connection is one of v. Müller's<sup>6</sup> aneurysm cases, in which the presphygmic period obtained from the cardiogram and aneurysmal pulsation was .04 to .05 second. Fourteen days later the aneurysm had increased in size and the cardiac force had diminished, and the pre-



Fig. 3.—Taken with the patient lying down. Shows a slight pulsus alternans and also alternate variations in the length of the  $V_s$ -S times.

sphygmic period had increased to .07 second. In our cases this relationship of  $V_s$ -S time to myocardial efficiency is perhaps best shown in the arrhythmias (Table 3), in which in each given case the  $V_s$ -S times vary according to the strength of the preceding systole and length of the preceding diastole, i. e., according to the degree of fatigue and recuperation of the heart muscle.



Fig. 4.—Case 21. Taken with the patient standing. Shows a marked pulsus alternans.

In the following case variations of the  $V_s$ -S time depend entirely on the strength of the preceding systoles.

CASE 21.—The patient was 54 years old; a painter by occupation. He gave a history of lues many years before, and of five attacks of lead colic. He had never had rheumatism. For two years he had been unable to do hard work because of palpitation, dyspnea and mild anginal pain.

The patient was not confined to bed, was not dyspneic or cyanotic, and was suffering no particular discomfort. Heart: The apex impulse was seen and felt in the fifth space, 11 cm. to the left of the mid-line. Dulness corresponded. No enlargement upward or to the right was made out by percussion. The first sound was loud, the second sounds were clear. There were no accentuations and no murmurs.

When the man was lying down the pulse was 80, of good size and force, at times regular and at times very slightly "alternans" in character. When he



TABLE 1.—CASES WITH REGULAR HEART ACTION, CLASSIFIED ACCORDING TO DIAGNOSIS

Case No.	Age	Diagnosis	Blood-Pressure	Pulse-Rate	S-R Time	V <sub>s</sub> -S or V <sub>s</sub> -C Time	P. P.	Speed per Meter
1	35	Normal heart .....	Normal	.....	.0950	.0890	.0740	.155
2	20	Normal heart .....	135/105	70	.0970	.0948	.0787	.146
3	20	Normal heart .....	Normal	75	.0860	.0960	.0823	.139
4	26	Aortic regurgitation	165/125	75	.0781	.0679	.0556	.137
5	.....	Aortic regurgitation	125/90	65	.1107	.0891	.0685	.190
6	37	Aortic regurgitation	170/115	63	.0764	.0847	.0687	.127
.....	.....	Same case standing	.....	75	.0759	.1100	.0962	.126
7	27	Aortic regurg. with great hypertrophy	.....	.....	.1000	.0870	.0690	.185
.....	.....	Same case standing	.....	.....	.0980	.1010	.0830	.181
8	46	Aortic regurgitation and stenosis .....	170/130	64	.0990	.0927	.0761	.165
9	19	Aortic regurgitation and mitral stenosis with relative regurgitation .....	120/90	80	.0860	.0870	.0730	.148
10	36	Aortic regurg. and mitral stenosis....	.....	70	.0954	.1006	.0836	.153
11	36	Aortic stenosis.....	105/70	45	.0674	.0920	.0813	.110
12	29	Mitral regurgitation	135/105	96	.0810	.0917	.0762	.147
13	40	Mitral regurgitation.	125/90	72	.1052	.1074	.0872	.166
14	37	Mitral regurgitation.	90/60	60	.0974	.1115	.0941	.157
15	17	Mitral regurgitation.	118/70	80	.0685	.1109	.1004	.118
16	26	Mitral regurgitation and stenosis .....	120/90	100	.0708	.1113	.0988	.131
17	42	"Beer heart." formerly acute dilatation; now compensated .....	190/170	60	.0664	.1175	.1061	.114
.....	.....	Same case standing	170/155	60	.0563	.1547	.1450	.097
18	32	Chr. diffuse neph.; cardiac hypertrophy .....	.....	100	.0568	.0849	.0759	.088
19	27	Probable patent ductus arteriosus....	.....	68	.0973	.0895	.0728	.153
20	60	Coronary sclerosis with ang. pectoris	120/75	85	.0732	.0826	.0714	.112
21	54	Myocarditis and arteriosclerosis; chronic plumbism; pulsus alternans....	155/120	80	.0826	.0828	.0676	.137
.....	.....	Same case standing	135/100	120	.0590	.1381	.1283	.098
22	17	Convalescent from acute tonsillitis....	95/75	68	.1260	.0735	.0504	.221

S-R Time = time taken by pulse wave to travel from subclavian to radial artery.

V<sub>s</sub>-S Time = Ventricular systole — subclavian interval.

V<sub>s</sub>-C Time = Ventricular systole — carotid interval.

P. P. = Presphygmie period.

stood up the pulse rose to 120, became markedly "alternans" and thus remained as long as the patient was standing. The radial artery was much thickened. For blood-pressures see Table 2.

The diagnosis was chronic plumbism, arteriosclerosis and myocarditis.

Tracings taken with the patient lying down (Fig. 3), showed a slightly alternating radial pulse, and a  $V_sS$  time which also alternated in length: being .0074 second shorter with the strong beats than with the weak ones; a difference which though very slight, was constant.



Fig. 5.—Case 21. Taken with the patient standing. Shows a marked pulsus alternans and great alternate variations in the length of the  $V_sS$  times.

Tracings taken with the patient standing (Figs. 4 and 5), showed a striking increase in these differences, for not only was the character of the radial pulse accentuated, but the  $V_sS$  time had an average alternation of .042 second, a variation more than five times as great as that obtained with the patient lying down. (Table 2.)

TABLE 2.—CASE 21. MYOCARDITIS AND ARTERIOSCLEROSIS; CHRONIC PLUMBISM; PULSUS ALTERNANS

Position	Blood Pressure		Pulse Rate		Average $V_sS$ time	Average P. P.	Difference
	Arm	Leg					
Lying	155/120	210/140	80	with weak beats	.0843	.0706	
Lying	155/120	210/140		with strong beats	.0769	.0632	.0074
Standing	135/100	250/?		with weak beats	.1591	.1493	
Standing	135/100	250/?	120	with strong beats	.1171	.1073	.0420

Table 2 shows a case with weakened contractility, in which the diastoles are equal, and the variations in the  $V_sS$  time depend entirely on the strength of the preceding systole. It is an interesting fact that the alternate variations of the  $V_sS$  time are greatly increased when the patient is standing. The mean lengthening which is present on standing, occurs to some degree in all cases and will be fully described later.

In this case, therefore, where there is every reason to suspect a heart muscle with impaired contractility, we have a  $V_sS$  time which alternates in length—the length evidently depending entirely on the amount of

force expended in the preceding systole. The diastoles in this case play no rôle, for, although at first sight they appear to alternate in length, they prove on actual measurement to be equal, the apparent variations being due to differences in the  $V_s$ -S time.<sup>18</sup>

TABLE 3.—SHOWING VARIATIONS IN THE  $V_s$ -S TIME

Case No.	Age	Diagnosis	Blood-Pressure	Pulse-Rate	Preceding Systole	Preceding Diastole	$V_s$ -S or $V_s$ -C Time	P. P.	S-R Time
23	25	Mitral regurgitation and stenosis, auricular fibrillation	125/90	90	S.	.272	.140	.128	.070
					W.	.184	.130	.116	.081
					W.	.187	.126	.113	.076
					S.	.322	.120	.108	.069
					X —				
					W.	.516	.096	.084	.072
					W.	.615	.096	.084	.068
					W.	.517	.096	.082	.080
					S.	.623	.090	.077	.078
					W.	.588	.086	.072	.084
24	60	Aortic regurgitation, arteriosclerosis, cardiac dilatation, auricular fibrillation	160/85	100	S.	.100	.200	.190	.053
					S.	.125	.171	.159	.060
					W.	.131	.159	.149	.048
					S.	.171	.158	.149	.048
					W.	.148	.141	.132	.046
					W.	.314	.131	.122	.051
					X —				
					W.	.526	.114	.104	.051
					W.	.761	.110	.099	.060
					W.	.432	.107	.098	.048
25	46	Myocarditis, auricular fibrillation	126/105	70	W.	.553	.106	.097	.048
					W.	.678	.102	.092	.051
					S. —	.381	.140	.128	.063
					S. —	.399	.132	.120	.066
					S. —	.473	.127	.116	.066
					S. —	.534	.127	.116	.064
					W.	.336	.126	.114	.068
					X —				
					S. —	.605	.105	.095	.056
					W.	.779	.092	.081	.066
26	47	Mitral regurgitation and possible mitral stenosis, auricular fibrillation		114	S. +	.310	.155	.140	.071
					W. +	.234	.138	.126	.071
					S. —	.402	.129	.118	.071
					W.	.322	.118	.108	.065
					W.	.314	.118	.106	.075
					X —				
					W.	.610	.105	.090	.075
					S. —	.736	.089	.077	.075

18. Diastoles have been determined by measuring from the lowest part of the dicrotic notch of the subclavian or carotid tracings to the following foot-point, and deducting the presphygmie period. The diastoles in this case average .218 second before the weak beats, and .210 second before the strong ones, this slight discrepancy being probably due to technical error. Without correction they alternate by a difference of .05 second.

TABLE 3—CONTINUED

Case No.	Age	Diagnosis	Blood-Pressure	Pulse Rate	Preceding Systole	Preceding Diastole	V <sub>s</sub> or V <sub>d</sub> Time	P.	R Time
27	72	Myocarditis and arteriosclerosis, auricular fibrillation.	210/160	90	S.	.220	.110	.100	.059
					S.	.220	.108	.098	.049
					W.+	.287	.101	.093	.048
					S.	.299	.100	.091	.051
					S.	.311	.098	.086	.053
					X	—	—	—	—
					W.	.330	.089	.080	.051
					S.	.784	.086	.076	.057
					S.	.435	.084	.075	.053
					W.+	.467	.082	.073	.050
					S.	.528	.082	.072	.059
					W.	.349	.080	.071	.053
					W.+	.360	.078	.070	.047
					—	—	—	—	—
28	69	Myocarditis and arteriosclerosis, auricular fibrillation	155/125	70	S.	.230	.096	.080	.090
					S.	.274	.082	.066	.090
					S.	.376	.079	.064	.080
					W.	.522	.072	.058	.082
					W.+	.425	.069	.055	.082
					W.	.505	.071	.055	.096
					W.	.566	.068	.054	.080
29	67	Mitral regurgitation, arteriosclerosis, auricular fibrillation	145/120	98	S.	.552	.065	.048	.098
					W.+	.194	.095	.086	.056
					S.—	.325	.084	.075	.055
					W.	.200	.080	.070	.059
					S.	.331	.078	.069	.055
					S.—	.373	.074	.067	.045
					S.	.273	.074	.066	.049
					S.—	.457	.071	.062	.051
					—	—	—	—	—

Table 3 shows marked variations of the V<sub>s</sub>-S time, depending on the length of the preceding diastole and the strength of the preceding systole.

In Cases 23 to 26, point "x" denotes the shortest diastole which seems to be required for the heart to regain its full power. Below this point, even with longer diastoles, the V<sub>s</sub>-S time varies but little.

The mean V<sub>s</sub>-S time is long, except in cases in which arteriosclerosis is a marked factor, as in Cases 27, 28 and 29.

The variation of the S-R time is largely due to technical error, which, on account of the extreme difficulty of getting sharp radial foot-points, is bound to be somewhat greater than in cases with regular heart action.

#### ARRHYTHMIAS

After much difficulty we obtained satisfactory tracings in seven cases of absolute arrhythmia. In each case the V<sub>s</sub>-S time varied widely in different curves, evidently depending on the degree to which the heart exhausted itself in systole, and in turn recuperated in diastole. Our



widest variation in any one case was from .102 to .200 seconds — the  $V_s$ -S time thus nearly doubling itself (Case 24). We found, as pointed out in regard to the presphygmie period by Robinson and Draper,<sup>1</sup> that the length of the  $V_s$ -S time seemed to depend on:

(a) The length of the preceding diastole; i. e., the shorter the diastole, the longer the  $V_s$ -S time.

(b) The strength of the preceding systole or systoles; i. e., the more force expended, the longer would be the following  $V_s$ -S time. This is shown in Table 3.

In Table 3 it is seen that as diastole grows shorter the  $V_s$ -S time rapidly lengthens. The opposite, however, seems to hold good only to a certain point. Some of our tracings strongly suggest that after a diastole of a certain length the heart has recovered its full ability, and that what slight shortening of the  $V_s$ -S time occurs after diastoles of still greater length may be due alone to further lowering of end diastolic pressure.

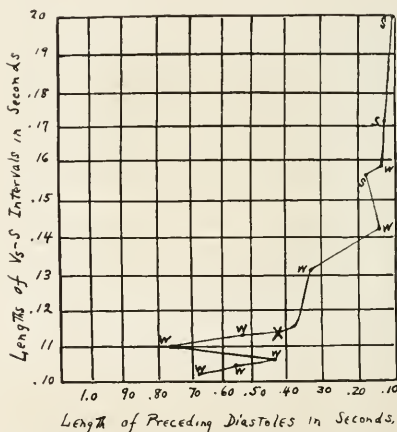


Fig. 6.—Case 24, showing relation of  $V_s$ -S time to length of preceding diastole and force of preceding systole. "S" denotes strong, "W" denotes weak preceding systole. Point "X" marks the shortest diastole which is apparently necessary for the heart to regain its full power. It is seen that to the left of this point the  $V_s$ -S time is fairly constant, whereas, to the right it varies widely, according to the length of diastole and strength of preceding systole.

For example, in Case 23 in Table 3, this point is seen to lie at "x"; i. e., somewhere between a diastole of .322 and .516 second. Above this line, following diastoles of less than .322 second, the  $V_s$ -S time varies widely as diastole and force of systole change; whereas, below the line, with diastoles above .516 second, they vary but little. Again, in Case 27, this point seems to lie between diastoles of .311 and .330 second. In Case 24 it lies between .314 and .432 second.

In all three of these cases, therefore, the time required for complete recuperation appears to lie between .311 and .526 second, and, if every heart were the same, we might place it between .322 and .330 second.

There can be little doubt, however, that different hearts vary considerably in this respect, as in the other cases we were unable to locate this point as definitely.

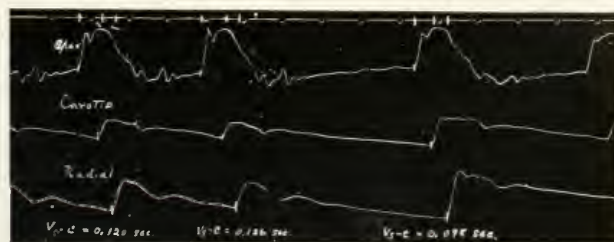


Fig. 7.—Case 23. Showing the influence of the preceding diastole on the length of the  $V_s$ -C time.

Curve 2.	$V_s$ -C=	.126 sec.	Diastole=	.311 sec.	C-R=	.068 sec.
Curve 1.	$V_s$ -C=	.120 sec.	Diastole=	.346 sec.	C-R=	.066 sec.
Curve 3.	$V_s$ -C=	.098 sec.	Diastole=	.776 sec.	C-R=	.068 sec.

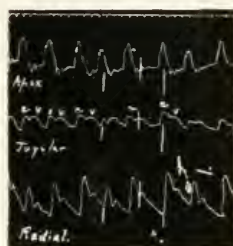


Fig. 8. Case 23. Venous tracing, showing absence of "A" wave.

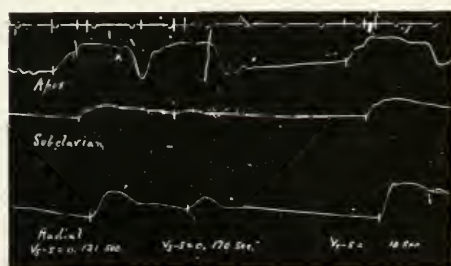


Fig. 9.—Case 24, showing the influence of the preceding diastole on the length of the  $V_s$ -S time.

Curve 2.	$V_s$ -S=	.170 sec.	Diastole=	.125 sec.	S-R=	.060 sec.
Curve 1.	$V_s$ -S=	.131 sec.	Diastole=	.314 sec.	S-R=	.051 sec.
Curve 3.	$V_s$ -S=	.110 sec.	Diastole=	.761 sec.	S-R=	.060 sec.

### *Influence of Posture on the $V_s$ -S Time*

To ascertain the effect of posture on the  $V_s$ -S time, tracings were first taken with the patient lying down. The patient then stood with his arm extended, so that the forearm lay at the level of the apex impulse: he

remained standing for ten minutes, when tracings were again made. We succeeded in getting satisfactory tracings of five cases. In every case the  $V_s$ -S time was definitely lengthened when the patient was standing (Table 4).

TABLE 4.—PATIENTS LYING AND STANDING

Case No.	Diagnosis	Blood-Pressure	Pulse-Rate	$V_s$ -S or $V_s$ -C Time	Difference	P. P.	Difference
17	"Beer heart," formerly acute dilatation, now compensated . . . . . (Same case standing) . . .	190/170	60	.1175	.....	.1061	.....
		170/155	60	.1547	.0372	.1450	.0389
21	Myocarditis and arteriosclerosis; chronic plumbism; pulsus alternans . . . . . (Same case standing) . . .	155/120	80	.0828	.....	.0676	.....
		135/100	120	.1381	.0553	.1283	.0607
7	Aortic regurgitation, with great hypertrophy . . . . . (Same case standing) . . .	.....	.....	.0870	.....	.0690	.....
		.....	.....	.1010	.0140	.0830	.0140
9	Aortic regurgitation and mitral stenosis (with relative regurgitation) . . . . . (Same case standing) . . .	120/90	80	.0870	.....	.0730	.....
		.....	80	.1020	.0150	.0880	.0150
6	Aortic regurgitation . . . . . (Same case standing) . . .	170/115	60	.0847	.....	.0687	.....
		.....	75	.1100	.0253	.0962	.0275

The  $V_s$ -S time is in every case longer in the erect than in the recumbent position. The greatest change in the  $V_s$ -S time is seen in Cases 17 and 21, which had no valvular lesions, but whose hearts were clinically considered to be the least competent. The possible prognostic value of this marked change in the  $V_s$ -S time is obvious.

It is interesting to note that the two hearts—Cases 17 and 21—which had no valvular lesions, but which we had reason to regard clinically as the least competent, show much the greatest lengthening of the  $V_s$ -S time (respectively, .0372 and .0553 second), whereas the hearts which, in spite of marked valvular lesions, seemed to have plenty of reserve force (Cases 6, 7 and 9), show a lengthening of only .014 to .025 second. Though we have reason to believe, from tracings obtained on normal cases standing, that a moderate lengthening of the  $V_s$ -S time constantly occurs, we were unfortunately not able to obtain figures which were accurate enough to record. The subject, however, should be further

investigated, for it is probable that a marked lengthening—such as is seen in Cases 17 and 21—might prove to be of great prognostic value.

Whether this lengthening of the  $V_s$ -S time is due to a change in the presphygmie period, or to delay of the pulse in the aorta, we are not prepared to say definitely, though we think that the presphygmie period is probably the main factor. The S-R time, though in three cases unaffected by posture, was in the other two somewhat shorter when the

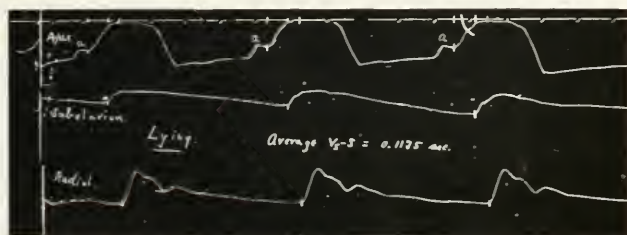


Fig. 10.—Case 17. Taken with the patient lying. Average  $V_s$ -S time .1175 second.

patients were standing. This means that, if there is any difference, the speed of the pulse in the arm is somewhat greater when standing than when lying down; and we would, therefore, not expect the speed in the aorta to be just the reverse. This point, however, is not at all certain.

As an explanation of the lengthening of the  $V_s$ -S time, we suggest that it is due to a sudden increase in the work imposed on the heart.

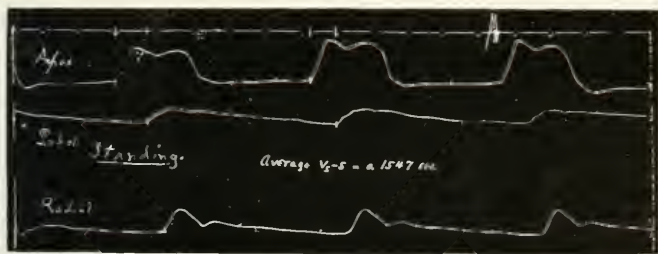


Fig. 11. Case 17. Patient standing. Shows marked lengthening of  $V_s$ -S time. Average  $V_s$ -S time now .1547 second.

Inasmuch as the venous column of blood from the level of the heart down, is heavier in the erect posture than the arterial column, as the experiments of Mosso<sup>19</sup> have shown it to be, it is obvious that, to maintain the circulation, the heart has to work against greater resistance. This would tend to lengthen the presphygmie period and  $V_s$ -S time, and especially would this be the case when a heart with little reserve was not quite equal

19. Mosso: Application de la balance a l'étude de la circulation du sang chez l'homme. Arch Ital. d. biol., 1884, v, 130.



to the task imposed on it. In terms of blood-pressure, this is equally true. Table 1 shows some relation in the length of the  $V_s$ -S time to diastolic pressure, and a fairly constant relation to pulse pressure, a low pulse pressure being associated with a long  $V_s$ -S time and *vice versa*. The careful investigation of the effect of posture on blood-pressure by

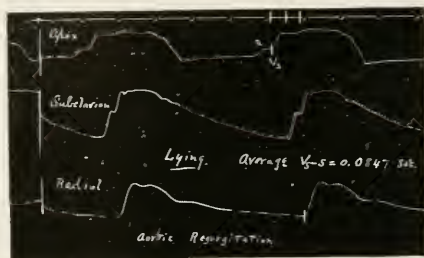


Fig. 12.—Case 6. Aortic regurgitation. Good compensation. Patient lying. Average  $V_s$ -S time = .0847 second.

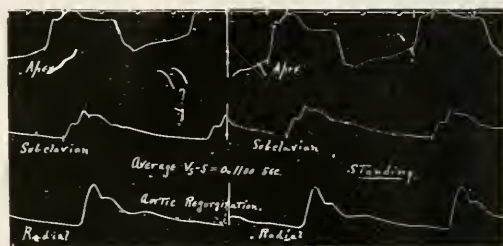


Fig. 13.—Case 6. Patient standing. Shows moderate lengthening of the  $V_s$ -S time, averaging .1100 second.

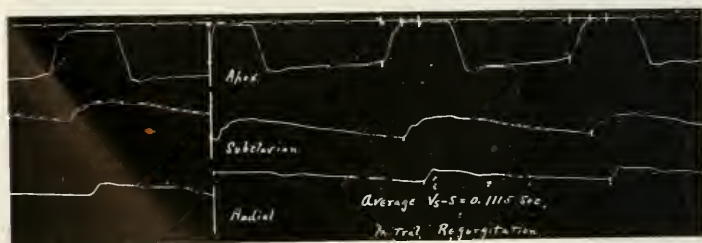


Fig. 14.—Case 14. Mitral regurgitation. Average  $V_s$ -S time = .1115 second. See also Tracing 11, Case 6, Aortic Regurgitation. Average  $V_s$ -S time = .0847 second.

Erlanger and Hooker<sup>20</sup> has shown that, when very accurate determinations are made, the diastolic pressure is usually higher, and the pulse pressure invariably lower in the erect than in the recumbent position. We should, therefore, rationally expect to find the  $V_s$ -S time lengthened, if affected at all, in the erect posture.

20. Erlanger and Hooker: Johns Hopkins Hosp. Rep., 1904, xii.

*The Effect of Valvular Lesions on the  $V_s$ -S Time*

Previous observers have differed as to the effect of various valve lesions on the  $V_s$ -S time and presphygmie period.<sup>21</sup> Our results go to show that, quite apart from other factors, aortic regurgitation tends to shorten and mitral lesions to lengthen the  $V_s$ -S time. Thus, in four cases of aortic regurgitation, the  $V_s$ -S times were either below or near the lower limits of normal, while in five mitral cases the times were all, with one exception, well above normal. The averages (see Table 5) show such striking differences that one is forced to believe that the  $V_s$ -S time is definitely affected by the nature of the lesion itself, as well as by the condition of the heart muscle.

TABLE 5.—CASES WITH REGULAR HEART ACTION: CLASSIFIED ACCORDING TO LENGTH OF  $V_s$ -S TIME

Diagnosis	No. of Cases	Average $V_s$ -S Time	Average P. P.
"Beer heart": formerly acute dilatation. Now compensated .....	1	.1175	.1061
Mitral regurgitation and stenosis .....	1	.1113	.0988
Mitral regurgitation .....	4	.1053	.0894
Aortic regurgitation and mitral stenosis .....	2	.0968	.0783
Normal heart .....	3	.0932	.0783
Aortic regurgitation and stenosis .....	1	.0927	.0760
Aortic stenosis .....	1	.0920	.0810
Probable patent ductus arteriosus .....	1	.0875	.0728
Chronic diffuse nephritis; cardiac hypertrophy .....	1	.0849	.0759
Myocarditis and arteriosclerosis; chronic plumbism; pulsus alternans .....	1	.0828	.0676
Coronary sclerosis and angina pectoris ..	1	.0826	.0714
Aortic regurgitation .....	4	.0822	.0654
Convalescent from acute tonsillitis .....	1	.0735	.0504

Table 5 shows the effect of valvular lesions on the  $V_s$ -S time. It is seen that in mitral cases the average is above normal, while in cases of aortic regurgitation it is below normal. In the cases of aortic stenosis and congenital heart disease, the lesion seems to have had little or no effect.

21. Keyt (Note 2) held that the  $V_s$ -C time was shorter in aortic regurgitation than in any other condition, except in fever cases with rapidly acting hearts, and that theoretically it should be lengthened in mitral lesions, although he had not estimated it in any such cases. Hilbert (Beitrag zur Deutung der Herzstosscurve, Ztschr. f. klin. Med., 1891, xix, Supplement, 153), on the other hand thought the presphygmie period unaffected by the lesion in hearts with good compensation. Hochhaus (Beiträge zur Cardiographie, Arch. f. exper. Path. u. Pharmacol., 1893, xxxi, 405; Ueber frustrane Herzkontraktionen, München. med. Wehnschr., 1907, liv, 401), found that in all his cases of mitral regurgitation and mitral stenosis the presphygmie period fell within normal limits. (He gives as normal .07-.10 second.) Robinson and Draper<sup>1</sup> concluded that the length of the presphygmie period depended more on the ability of the heart to meet demands than on the lesion itself.

*Minor Points Regarding the  $V_s$ -S Time*

A marked degree of arteriosclerosis tends to shorten the  $V_s$ -S time. This observation was made by Robinson and Draper<sup>1</sup> in regard to the presphygmic period. Our cases have confirmed this, as is shown by Case 21 in Table 1, and by Cases 27, 28 and 29 in Table 3.

We found that the  $V_s$ -S time bore no constant relation to pulse rate.<sup>22</sup>

Though the effect of blood-pressure is not as constant as we might expect, our cases showed some relation of the  $V_s$ -S time to diastolic pressures; and this relationship was most definite in cases with a marked difference between the systolic and diastolic pressure (high pulse pressure) in which cases we nearly always obtained a short  $V_s$ -S time.

One might expect that a split "C" wave in the jugular tracing would be associated with a long  $V_s$ -S time and presphygmic period. Inasmuch as the first part of the split wave — the "VK" wave of the Germans —

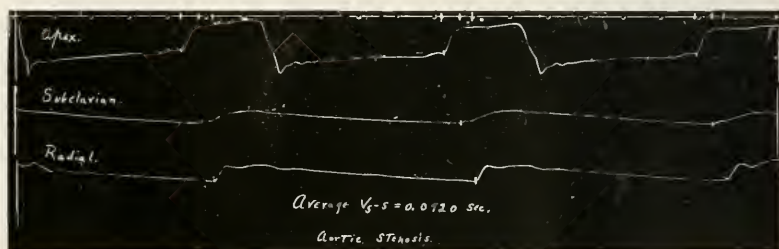


Fig. 15.—Case 11. Aortic stenosis. Average  $V_s$ -S time = .0920 second.

is probably due to impact on the tricuspid flaps exerted in early ventricular systole, and is separated from the opening of the aortic valves by a portion of the presphygmic period, we should expect, in the case of a long presphygmic period, to have a well-marked "VK-C" interval. In none of our cases, however, which showed a split "C" wave in the jugular tracing, was there a  $V_s$ -S time or presphygmic period above normal. (See Table 6.)

## CONCLUSIONS

1. The normal  $V_s$ -S time is from .085 to .10 second. It varies widely in certain abnormal conditions of the heart and circulation.

2. The presphygmic period, as determined by calculation from simultaneous tracings of the apex beat, subclavian and radial pulses, is about .015 second shorter than the  $V_s$ -S time, and runs parallel to it.

22. Though Carrod (Note 12) thought that the presphygmic period was short in cases with a rapid pulse rate and *vice versa*, our cases failed to show any constant relationship between the pulse rate and the  $V_s$ -S time. Our observations agree with those previously made in regard to the presphygmic period by Hürthle (Note 9) and by Robinson and Draper (Note 1).

variations in one fairly accurately representing variations in the other. We doubt the accuracy of the presphygmie period as obtained by this method and hope to settle this point more definitely in future work.

3. In cases of auricular fibrillation the variations in the  $V_s$ -S time depend on: (a) the length of the preceding diastole, and (b) the strength of the preceding systole or systoles: being longest after a short diastole, and a strong systole, and *vice versa*. After diastoles of a certain length, however, the length of the  $V_s$ -S time varies little or not at all. From figures obtained in three cases, the length of diastole necessary for complete rest apparently lay between .322 and .330 second.

4. In cases of broken compensation, the mean  $V_s$ -S time was invariably lengthened, except when a marked degree of arteriosclerosis was present. It is probable that, when no disturbing factors coexist, the length of the  $V_s$ -S time may be of value in estimating the capability of the heart muscle.

TABLE 6.—SHOWS THAT A SPLIT "C" WAVE IN THE JUGULAR TRACINGS WAS NOT ASSOCIATED WITH A LENGTHENED  $V_s$ -S TIME

Case 1.	Average VK-C interval = .032	$V_s$ -S = .0890	P. P. = .0740
	Average A -C interval = .205		
Case 8.	Average VK-C interval = .012	$V_s$ -S = .0927	P. P. = .0761
Case 12.	Average VK-C interval = .029	$V_s$ -S = .0917	P. P. = .0810
	Average A -C interval = .181		
Case 19.	Average VK-C interval = .053	$V_s$ -S = .0895	P. P. = .0728
	Average A -C interval = .277		

5. In every one of five cases on which tracings were taken with the patients lying and standing, the  $V_s$ -S time was definitely longer in the erect posture. As by far the greatest lengthening occurred in the two cases which were clinically regarded as having the least competent hearts, it is probable that the degree of lengthening may prove to be of value in prognosis.

6. Aortic regurgitation shortens, and mitral valve lesions lengthen, the  $V_s$ -S time. Combined lesions, having opposite effects, tend to offset one another.

7. A marked degree of arteriosclerosis shortens the  $V_s$ -S time.

8. The relation of the  $V_s$ -S time to blood-pressure is not constant. It tends, however, to become shortened when the pulse pressure is high, especially if at the same time, the diastolic pressure is low. The opposite also holds good.

9. Split "C" waves in the jugular tracings were not associated with abnormally long  $V_s$ -S times.

10. The speed of the pulse wave in the arm varies with extremes of blood-pressures, being faster in cases with high, and slower in cases with low, blood-pressure. This relationship, however, is not constant. No



other factors seem to have any definite effect on the pulse speed. Although it varies widely in different individuals, it is nearly always constant in a given vessel of the same individual.

In conclusion we wish to express our thanks to Professor Friedrich v. Müller for the splendid material afforded us, and to Dr. Ernst Edens for his aid in the interpretations of tracings and for many helpful suggestions. We also wish to thank the assistants of the Second Medical Clinic for many courtesies extended to us in the wards.

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## THE RELATION OF THE HYPOPHYSIS TO GROWTH AND THE EFFECT OF FEEDING ANTERIOR AND POSTERIOR LOBE \*

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CHICAGO

As observed by the clinician, there are two clinical entities, both disturbances of growth, ascribed to disturbed functioning of the hypophysis. One is acromegaly, characterized by enlargement of certain bones; the other, delayed development with adiposity and genital atrophy. When the former occurs in early life, gigantism results; when it first appears after maturity, enlargement of only certain portions of the bony skeleton is observed. In the other type, when the disturbance appears in early life, there is delayed skeletal development, with adiposity and failure of sexual evolution; when it appears after maturity, adiposity and sexual atrophy.

Pierre Marie, in 1886, first called attention to the relation between acromegaly and the hypophysis, although Carl von Langer in 1872, in an anatomical study of giants, referred to a certain type with enlarged sella turcica. The observations of Marie have been confirmed, until at present it is generally conceded that acromegaly is due to a disturbance of the hypophysis. According to Sternberg, 40 per cent. of the pathologic giants have an enlargement of this organ. Regarding the exact nature of the disturbance in the hypophysis in acromegaly, there is still considerable difference of opinion. The weight of evidence, however, favors the view that it is due to hypersecretion of the anterior lobe. The pathologic condition most frequently associated with acromegaly is an adenomatous development of the anterior lobe with an increase in the specific secretory cells. In some instances in which enlargement of the anterior lobe is lacking, increase in the specific secretory cells may still be demonstrated, and in addition hyperplasia of the pharyngeal hypophysis should be considered in these cases. There are reported in the literature, malignant tumors of the hypophysis with acromegaly. Lewis, who has reviewed these cases, believes that in the majority, and possibly in all, of these the tumor was an adenoma. Although it is too early to state positively that acromegaly is due to hypersecretion from the anterior lobe, it must be admitted that the weight of evidence supports this view.

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\* From the Department of Experimental Therapeutics, University of Chicago, S. A. Matthews, director.

\* Presented at the Eighth International Congress of Applied Chemistry.

The condition of underdevelopment, adiposity and genital atrophy, first described by Fröhlich and referred to as the Fröhlich syndrome, is usually associated with disturbances at the base of the brain, more especially with tumors. Whether in all of these cases the hypophysis is involved, either directly or indirectly by either increased intracranial or intracerebral pressure, has not been determined. On account of its position, the hypophysis is especially liable to be influenced when there is an internal hydrocephalus, and it is quite possible that any cerebral disturbance causing increased pressure in the ventricle may compress and disturb the hypophysis. There is one case on record in which a bullet wound of the hypophysis was followed by adiposity (Madelung). Adiposity is associated with acromegaly, according to Creutzfeldt, in 1.6 per cent. of cases. In five of the recorded cases of adiposis dolorosa, with autopsy, the hypophysis has been abnormal (Lyon). Cases are also on record in which removal of the hypophyseal tumor was followed by disappearance of the adiposity (Von Eiselberg).

Assuming that adiposity may arise from disturbances of the hypophysis, it is still to be determined what portion of the glands is responsible for these changes. Fischer believes it is due to hyposecretion of the posterior lobe. In addition to certain theoretical considerations, he presents some autopsy findings in which as the result of pressure, the posterior lobe was flattened and had undergone brown atrophy. It can readily be conceived, however, that any pressure involving the posterior lobe must also compress the anterior portion. In all of Fischer's evidence there is little that is actually convincing. A more tenable view is that hyposecretion of the anterior lobe is responsible for the adiposity. Zollner, in a case of this type, found a carcinoma of the anterior lobe. In the five cases of adiposis dolorosa, referred to, with hypophyseal involvement, two showed round-cell infiltration of the anterior lobe (Price), one a glioma evidently of the posterior lobe (Burr), one marked increase in size of the anterior lobe, due to a connective tissue hyperplasia and an alveolar sarcoma (Guillain), and one (Dercum's case) a carcinoma involving both anterior and posterior lobes. Although few deductions may be drawn from the above evidence, on the whole it favors disturbance of the anterior lobe. Most convincing, however, is the experimental evidence. In dogs, removal of the posterior lobe is not followed by any serious consequence, the animal recovers and later fails to show any anomalies of growth (Paulesco, Cushing, Ascola). Where a considerable portion of the anterior lobe of a young dog is removed and the animal recovers from the effect of the operation, he later develops the Fröhlich syndrome of delayed development, adiposity and failure of sexual development (Aschner, Cushing, Casselli, Ascola). This evidence is most suggestive and points strongly toward hyposecretion of the anterior lobe

as the cause of the adiposity and sexual atrophy. The question may properly be raised whether the adiposity is due directly to the lessened secretion of the anterior lobe or is secondary to the genital atrophy, for Tandler and Grosz have shown that castration in dogs leads to increased deposits of fat, and this phenomenon is observed in women after removal of the ovaries. It might be argued that it would be exceedingly difficult on the basis of hyposecretion of the anterior lobe to explain the occasional presence of adiposity in acromegaly. Such adiposity is very infrequent, occurring, according to Creutzfeldt, in only 1.7 per cent. of cases, while hypoplasia and genital atrophy were noted in 36.4 per cent. of the cases. The greater frequency of the sexual disturbance would lead us to suspect that it and the adiposity were due to separate factors. Here, again, the possibility of the adiposity being secondary to the genital atrophy must be considered, its inconstant associations being due to the varying degrees of sexual hypoplasia in the various cases. To those who believe that the adiposity is due to the hyposecretion of the posterior lobe, its presence in acromegaly is explained by pressure of the enlarged anterior lobe on the posterior lobe.

Summarizing, it may be said that acromegaly is probably due to increased activity of the anterior lobe. Adiposity, if due directly to disturbances of the hypophysis, is most probably due to hyposecretion of the anterior lobe.

#### METABOLISM IN ACROMEGALY

The metabolism in acromegaly has now been studied in a dozen or more cases. Retention of nitrogen is very frequent, often reaching considerable amounts. In some of these cases calcium and phosphorus metabolism is unchanged; in others, retention of both calcium and phosphorus have been reported. Seven cases from five different observers all show a retention of nitrogen, five of phosphorus and five of calcium. Oberndörffer has recently reported two cases and reviewed the literature on this subject. He was unable to detect any variation from the normal in his two cases, and questions whether the results of others are really conclusive, on account of the great variations in elimination of normal individuals. Before the work on this phase of the subject can be accepted, more extensive studies should be carried out.

#### METABOLISM IN ANIMALS AFTER THE ADMINISTRATION OF HYPOPHYSIS

Thompson and Johnson fed dogs on the entire dried gland from the horse, calf and sheep, and found that they lost in weight and excreted an excessive amount of nitrogen and phosphorus (ca. and mg. not determined). They also reported more marked results when glands of young animals were used. Malcomb gave dogs 2 to 3 gm. daily of dried



anterior lobe, for a period of five days, a total of 15 gm. The animals showed slight retention of nitrogen and slightly increased output of  $P_2O_5$  and Ca. After feeding posterior lobe (10 gm.) there occurred a slightly increased output of  $P_2O_5$  and marked increased output of Ca. When he gave fresh entire glands, 25 gm. daily, there was scarcely any change in the Ca or Mg. output. Franchini injected rabbits, intravenously, daily with an amount of extract equivalent to one entire hypophysis. This was followed by greater elimination of Ca, Mg. and  $P_2O_5$  in both urine and feces, the loss in  $P_2O_5$  being less marked than of Ca or Mg. The animals lost in weight and finally died. The x-ray failed to show any change in the bony skeleton. Some of the animals tolerated the injection well; others showed marked dyspnea, vomiting and diarrhea. Franchini's conclusions that hyperpituitarism leads to loss in weight and failure of development is scarcely justified on account of the severe reaction following his intravenous injections. Oswald gave dogs 2 to 3 gm. daily of dried hypophyseal extract obtained from Merck (portion of gland not specified) and was unable to detect any change in nitrogen or  $P_2O_5$  elimination. Benedict and Homans, working with hypophysectomized dogs and using carbon dioxide production as an index of total metabolism, found it markedly reduced.

The results of these findings vary so much that they throw little light on the disturbance of metabolism following administration of dried hypophysis and may be regarded as furnishing no definite evidence.

#### FEEDING EXPERIMENTS

Comparatively few satisfactory feeding experiments have been reported. In some, no attempt has been made to feed the two lobes separately. Others have administered the extract subcutaneously or intravenously (Cerletti, Franchini, Delille, Caselli). When given in this way it frequently gives rise to marked constitutional disturbances, such as vomiting and diarrhea, and, finally, intestinal ulceration, that it is impossible to draw any conclusion regarding the actual effect of the hypophyseal extract. After this method of administration, Cerletti and Franchini report loss in weight and delayed bone development. The entire hypophysis was used and the animals treated for a few days only. Caselli injected young dogs and rabbits with glycerin extracts and did not notice any effect on growth. Delille injected extracts of the entire hypophysis into four rabbits for a period of fourteen months and reported increased deposits of fat.

Only two references to feeding experiments have been obtained in which the animals received preparations of the hypophysis by mouth for a considerable period of time.

Sandri fed rats on hypophysis exclusively for a period of two months, the controls receiving an exclusive meat diet. While this is an unsuitable

diet, Sandri reports that the animals thrived. He found that those fed on the anterior lobe showed greater gain in weight than the controls. When we consult the actual figures, we find that these differences are so slight that they can scarcely be considered as significant. The controls during the two months gained, on an average, 10 gm.; those fed on the posterior lobe, 7 gm.; those fed on the anterior lobe, 12 gm. Variation of this degree may occur in any group of feeding experiments continued over a period of three months.

Schaefer has conducted the most satisfactory feeding experiments, using, however, only the anterior lobe. Four young rats were fed small amounts of the dried anterior lobe, mixed with bread and milk. The controls received powdered testicle or ovary, with bread and milk. The amount consumed by each group of animals was accurately determined. The feeding experiment was continued for about three months. At the beginning, the average weight of the group fed on hypophysis was 44.25 gm., and that of the controls exactly the same. At the end of the feeding, the average weight of those fed on hypophysis was 160 gm., and of the controls, 131 gm. During the first six weeks of the feeding, there was little difference between the two groups; during the last six weeks those fed on hypophysis made the more rapid gain. These results would appear to be conclusive; they are not, however, sufficiently numerous to eliminate error.

#### AUTHORS' EXPERIMENTS

In undertaking this investigation, it was decided to carry through several series of animals with controls. Young white rats were selected. Each rat was placed in an individual cage. For food, ground cracker was chosen. This was pressed into tablets, each of the same weight. It was first determined how much of this food each rat would consume daily. Although there were some individual differences, it was possible to determine with reasonable accuracy the daily ration. Having determined this point, cracker tablets of the requisite weight were made, and to each was added a weighed amount of the hypophysis, or in case of the control, meat, and each animal received the same amount daily. Occasionally, for a few days, a rat might not eat his whole tablet; a note was made of this fact. However, the ration was so arranged that, with rare exceptions, it was consumed daily. No doubt some of the animals would have eaten more. The fact that they gained in weight and appeared on the whole healthy would, however, indicate that they were properly fed. By this method, each rat received and consumed the same amount of food daily, containing the same amount of the substance to be tested. The animals were weighed each week.

The ox hypophyses were obtained perfectly fresh from the Union Stock Yards. The anterior and posterior lobes were separated, chopped

up fine and dried in a blower at a temperature of approximately 100 F. The dried glands were then powdered and a weighed amount added to the powdered cracker and pressed into a tablet. Three series were fed in this way for about three months each; at the end of the time the rats were killed and radiographs taken to detect any changes in the bony skeleton. The first series consisted of nine rats. Three received daily 2 gm. of dried anterior lobe, three the same amount of posterior lobe and three controls the same amount of meat. The feeding was continued for seventy-nine days. The second series consisted of eight rats. Four received 4 gm. anterior lobe and the other four as controls received the same amount of dried meat. This group was fed for ninety days. The third series of nine young rats was divided into three groups. One group received 3 gm. daily of beef, another the same amount of posterior lobe, and the other the same amount of dried thymus. This series was kept under observation for sixty-seven days. By repeating the experiment in this manner, it was thought that some sources of error might be eliminated.

TABLE 1.—RESULTS OF FEEDING EXPERIMENTS IN RATS

Number of Animals	Food Daily, Gm.	Average Weight at Beginning, Gm.	Average Weight at Termination, Gm.	Change in Weight, Gm.	Period of Feeding, Days.
SERIES I					
3	Dried beef, .2.....	52.2	91.8	38.6	78
3	Dried anterior lobe, .2.....	54.2	92.3	37.9	78
3	Dried posterior lobe, .2.....	58.1	102.6	44.5	78
SERIES II					
4	Dried beef, .4.....	58	95.2	37.2	90
4	Dried anterior lobe, .4.....	66.6	107.3	40.7	90
SERIES III					
3	Dried beef, .3.....	131.6	144.3	12.6	67
3	Dried posterior lobe, .3.....	115.3	121.5	6.2	67
3	Dried thymus, .3.....	118.6	135.3	16.7	67

It is interesting to note that animals consuming the same amount of food daily and apparently enjoying equally good health, should show such marked variations of gain in weight. The minimum gain in weight of the controls in Series I was 30.6 gm., the maximum, 44.4 gm. In Series II the minimum gain in weight of the control rats was 32 gm., the maximum 38.5 gm. In Series III, wherein the rats were two-thirds grown, and, therefore, not so suited for the test, in both the controls and those fed on posterior lobe, one of the animals lost 5 gm.

As will be seen by the table in the first series, the controls and those fed on the anterior lobe showed practically the same gain in weight. Those fed on the posterior lobe, gained an average of 6 gm. each more than the controls. When we consider the individual animals, one of those fed on the posterior lobe gained less than one of the controls, the other two gained more than the controls and each animal fed on the posterior lobe gained more than those receiving the anterior lobe. In Series III, however, the animals receiving the posterior lobe gained less than the controls and much less than those animals receiving thymus. In Series I, animals receiving anterior lobe gained slightly less than the controls, while in Series II, they gained somewhat more than the controls. The radiographs of all these animals failed to reveal any variations in the bony skeleton.

Only one conclusion can be drawn from these feeding experiments, viz., that, at least in this series of tests, neither anterior nor posterior lobes had any effect on the weight or growth of the animal. The experiment was conducted in such a manner that serious causes of error were excluded. The amounts administered were sufficient to give results, as they would be equivalent to 230 gm. daily to the average man. On the other hand, the amounts were not sufficiently large to have a deleterious effect, as the animals so fed gained the same in weight as the controls. Doubling the dose of anterior lobe did not modify results. It must be admitted, however, that this does not prove that disturbed secretion of the hypophysis may not modify growth. In the feeding experiment, the digestive fluids may destroy the active substances responsible for these changes. Again, feeding preparations by mouth can scarcely be considered as analogous to the continuous secretion occurring in actual life.

#### SUMMARY

Summarizing the entire field of the rôle of the hypophysis in the growth of the individual in acromegaly in which there exists abnormal development of certain portions of the body, especially in their bony structures, there is apparently hypersecretion of the anterior lobe. In the Fröhlich syndrome of adiposity and failure of sexual development, it is thought by many that there is lessened function of the posterior lobe; experimental information, however, suggests lessened secretion of the anterior lobe, but evidence on this point is not especially convincing. Regarding studies in metabolism in patients with acromegaly, there is again nothing conclusive, and more work must be carried out on this subject before it can be accepted that lessened katabolism takes place as compared with the normal individual.

Turning to the results of partial removal of the hypophysis in animals, only one point having a direct bearing on this subject seems to



have been determined, viz., that partial removal of the anterior lobe, when performed on young animals, modifies growth and sexual development in such a manner as to resemble very closely the Fröhlich syndrome. Removal of the posterior lobe, apparently, has no effect on growth. This is a distinct contradiction to those who believe lessened function of the posterior lobe is responsible for the Fröhlich syndrome. Feeding experiments, on animals, fail to furnish any definite evidence that the administration of either the anterior or posterior lobe has any effect on growth.

We wish to express our thanks to Mr. E. M. Miller for his assistance in this investigation.

Since this work was completed, T. B. Aldrich under the title, Feeding Young Pups the Anterior Lobe of the Pituitary (*Am. Jour. Physiol.*, 1912, xxx, 352), has reported negative results after feeding anterior lobe.

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# A STUDY OF HYDROGEN ION CONCENTRATION OF THE URINE IN HEART DISEASE \*

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The circulating fluids of the organism are practically neutral and nearly constant in reaction, and an animal, into whose body acid is being introduced, dies before his serum gives an appreciably acid reaction.<sup>1</sup> Nevertheless, the trend of metabolism is toward the production of acid greatly in excess of alkali; but under normal conditions the whole of this excess is separated from the blood in its passage through the kidney. One of us has shown the chemical mechanism which underlies

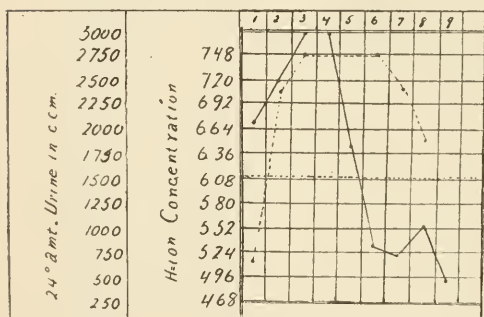


Chart 1.

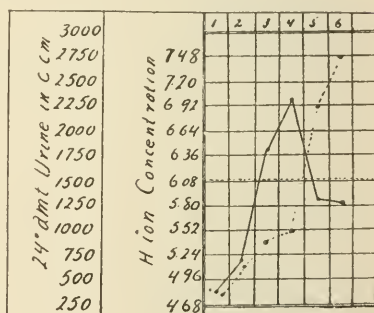


Chart 2.

Chart 1.—Case 1. Double hydrothorax; large ascites; marked general anasarca; arteriosclerosis; cardio-renal. Blood-pressure 160. Made a rapid and uneventful recovery.

Chart 2.—Case 2. Mitral disease. No hydrothorax, ascites or edema. Moist râles in lungs. Improved rapidly.

The solid line in this and other charts represents the twenty-four hour amount of urine in cubic centimeters. The broken line represents the hydrogen ion concentration in each twenty-four-hour specimen of urine expressed logarithmically. The horizontal broken line represents the mean of the hydrogen ion concentration of the twenty-four-hour specimens of urine from 150 normal persons.

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\* Submitted for publication May 1, 1913.

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1. Walter, F.: Untersuchungen über die Wirkung der Säuren auf den thierischen Organismus, Arch f. exper. Path., 1877. vii. p. 148.

this neutrality preservation and has pointed out its far-reaching biological significance.<sup>2</sup>

Whether the acid can be removed as fast as it is produced in various abnormal conditions, and, the mechanism failing, what pathological states

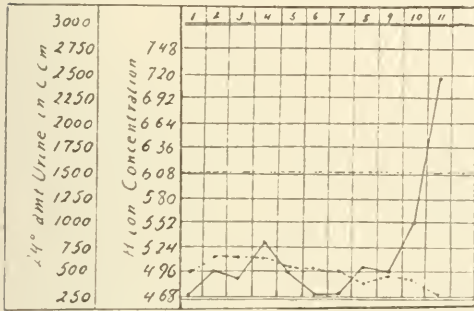


Chart 3.

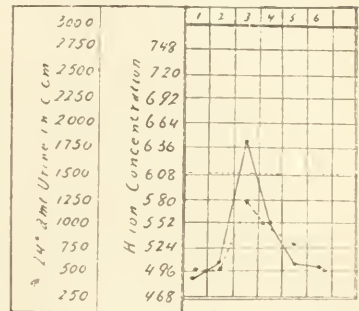


Chart 4.

Chart 3.—Case 3. Arteriosclerosis; weak dilated heart; marked edema of legs. Patient became subjectively comfortable but objective signs did not change.

Chart 4.—Case 4. Chronic nephritis. Blood-pressure 250; right hydrothorax; marked edema of legs; edema disappeared at time of diuresis. Otherwise no improvement.

may be thereby called into existence, are matters still open to investigation.

In order further to investigate this question, it seemed advisable in the first place to study the reaction of the urine when disturbance of the

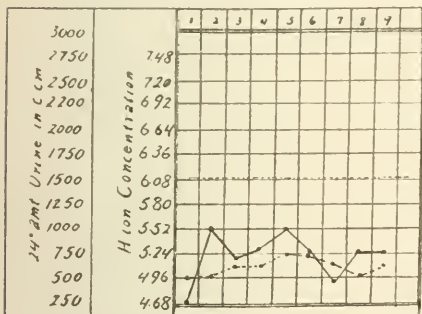


Chart 5.

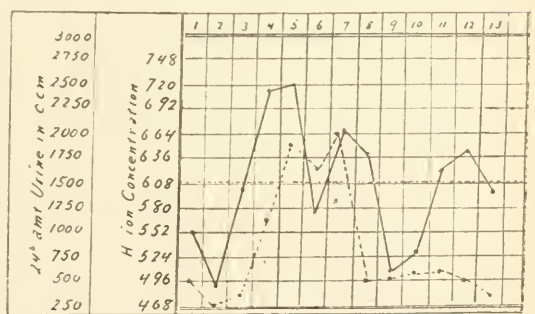


Chart 6.

Chart 5.—Case 5. Syphilitic aortitis. Aortic regurgitation; marked edema of legs. Made no improvement.

Chart 6.—Case 6. Arteriosclerosis. Weak dilated heart; double hydrothorax, marked edema of legs; marked improvement during period of diuresis with disappearance of edema then stationary. Discharged with compensation only partly restored.

acid excretory function might be suspected. It was believed that an abnormally large amount of acid in the urine would perhaps indicate the *overproduction* of acid in the organism, or partial failure of the protective mechanism.

2. Henderson, L. J.: The Theory of Neutrality Regulation in the Animal Organism. *Am. Jour. Physiol.*, 1908, xxi, 427.



The work to be reported in this communication is concerned only with urinary acidities in persons suffering from cardiac decompensation.

It is now well known that the true acidity of any solution must be expressed in terms of the concentration of ionized hydrogen; and that in complex solutions like urine, acidity determined in this way may give results which vary greatly from acidity determined by titration against

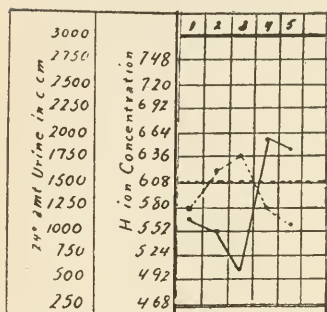


Chart 7.

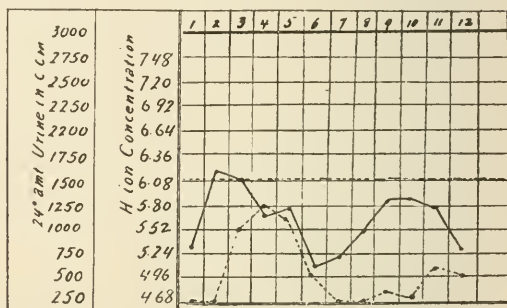


Chart 8.

Chart 7.—Case 7. Mild break. No edema. Rapid recovery.

Chart 8.—Case 8. Myocardial weakness. Marked dyspnea and orthopnea; weak, irregular pulse; no hydrothorax or ascites; very slight edema of legs. He improved rapidly at first, then became stationary, being unable to make any exertion without return of dyspnea.

alkali. In order to determine hydrogen ion concentrations in the clinical laboratory, some reasonably simple method had to be devised. This has been accomplished by two of us<sup>3</sup> and that method employed in this study.

For the convenience of the many clinicians who have not yet become familiar with the physico-chemical mode of expressing acidity, a brief statement will be added. Acidity is always thought of as the number of grams of ionized hydrogen in a solution when these are in excess of the hydroxyl ions. Since the

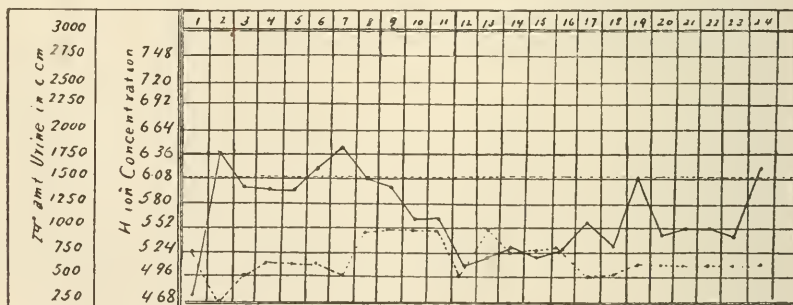


Chart 9.—Case 10. Aortic and mitral disease. Double hydrothorax; ascites; marked general anasarca; improved slowly; discharged with slight edema; otherwise no evidence of broken compensation.

3. Henderson, L. J., and Palmer, W. W.: On the Intensity of Urinary Acidity in Normal and Pathological Conditions. *Jour. of Biol. Chemistry*, 1913, xiii, 393.

purest water thus far obtained is slightly ionized and thus contains hydrogen ions (which, in the case of water, are in concentration equal to the hydroxyl ions) a solution, to be acid, must contain more hydrogen ions per liter than those resulting from the water, and at the same time, the hydrogen ions must be in excess of the hydroxyl ions. The ionization of hydrogen in water is actually  $1/10,000,000$  gram per liter at  $25^{\circ}\text{C}$ ., or, otherwise expressed,  $1 \times 10^{-7}$ . Consequently the condition of acidity may be expressed as a formula thus:

$$\frac{+}{(\text{H})} > \frac{+}{1/10,000,000 \text{ N}} > \frac{-}{(\text{OH})}.$$

Neutrality is then

$$\frac{+}{(\text{H})} = \frac{+}{1/10,000,000 \text{ N}} = \frac{-}{(\text{OH})}.$$

And alkalinity

$$\frac{+}{(\text{H})} < \frac{+}{1/10,000,000 \text{ N}} < \frac{-}{(\text{OH})}.$$

$\frac{+}{(\text{H})}$  = the concentration of ionized hydrogen per liter expressed in the usual way.

$\frac{-}{(\text{OH})}$  = the concentration of hydroxyl was per liter and  $1/10,000,000$  the concentration of the ions at the neutral point.

Since the ionization of water and of hydrogen and hydroxyl in solutions found in animal organisms is relatively so slight, the number of grams of hydrogen ions per liter is actually exceedingly small. It is consequently much more satisfactory to express the results as the logarithms of the number. Thus for water—7.00 instead of  $1/10,000,000$ . (As the numbers increase the acidity decreases; as the numbers decrease the acidity increases.) The minus sign is omitted for convenience.<sup>4</sup>

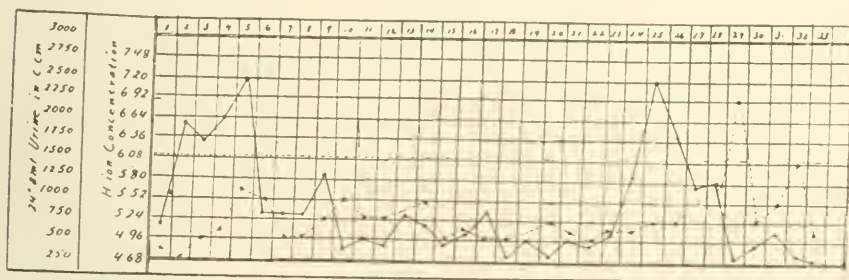


Chart 10.—Case 11. Mitral stenosis. Enlarged liver; edema of lungs; anasarca. During first period of diuresis there was not much clinical improvement, then came a period when condition was no better than at entrance. The second period of diuresis followed venesection and strophanthin intravenously, and was attended by marked clinical improvement, but the latter was only of several days' duration and the patient was finally discharged no better than at entrance.

The data here presented are derived from the daily examination of the twenty-four-hour specimens of urine of fifteen patients with "cardiac edema." The decompensation varied from the severest grade to the very mild type. The cases may be divided into two groups: (1) Degenerative, including arteriosclerosis with myocardial changes, and myocardial weak-

4. Further details concerning this physical-chemical conception of acidity will be found in Palmer and Henderson's paper in this issue p. 153: Clinical Studies in Acid Base Equilibrium and the Nature of Acidosis.

ness following interstitial nephritis. Cardiac failure occurring with glomerular nephritis was excluded. (2) Infectious, by which is meant injury to the valves dependent on microbic invasion with secondary failure of compensation. All active (malignant endocarditis) cases were excluded. The patients varied in age from 12 to 62 years.

The severe cases, eleven in number (Cases 1, 2, 3, 4, 5, 6, 8, 10, 11, 12, 14, see charts), had, on admission and up to the time when improvement began, a hydrogen ion concentration which averaged 4.90. The lowest single reading during this period was 5.30, the highest 4.70. The two cases with mild breaks in compensation (Cases 7 and 15, see charts) averaged 5.70, and the highest single reading was 5.30. In a study of the hydrogen ion concentration of the urines of one hundred and fifty

Table showing the hydrogen ion concentration of the twenty-four hour specimens of urine in eleven cases of severe cardiac decompensation, from admissions up to the time when definite improvement occurred. Cases 1 and 12 began to improve almost immediately after admission.

Case	Days											Average
	1	2	3	4	5	6	7	8	9	10	11	
1...	5.15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	5.15
2...	4.85	5.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.92
3...	5.00	5.15	5.15	5.15	5.00	5.00	5.00	4.85	5.00	4.85	4.70	5.00
4...	5.00	5.00	5.00	.....	.....	.....	.....	.....	.....	.....	.....	5.00
5...	5.15	5.00	5.00	5.15	5.15	5.30	5.30	5.15	5.00	5.15	.....	5.13
6...	4.85	4.85	4.70	4.85	.....	.....	.....	.....	.....	.....	.....	4.81
8...	4.70	4.70	4.70	.....	.....	.....	.....	.....	.....	.....	.....	4.70
9...	4.70	5.00	5.15	5.15	5.15	5.00	.....	.....	.....	.....	.....	5.02
11...	4.85	4.70	5.00	.....	.....	.....	.....	.....	.....	.....	.....	4.85
12...	4.85	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.85
14...	5.00	5.00	5.00	.....	.....	.....	.....	.....	.....	.....	.....	5.00

normal persons,<sup>3</sup> we have found the normal average to be 6.00. In less than 1 per cent. of these normal cases was the hydrogen ion concentration greater than 5.00, and in less than 10 per cent. was it greater than 5.30. Thus it is clear that persons with severe heart disease produce urine whose hydrogen ion concentration is distinctly above the normal.

If the hydrogen ion concentration is followed in reference to the clinical course of the cases (see figures), it is found that the former decreases as the individuals recover compensation (Cases 1, 2, and 14), and that in the individuals who have not improved markedly, the hydrogen ion concentration remains continuously high (Cases 3, 5, 8, 10, 11 and 12).

The next point to be considered is whether there is any relation between the edema and the hydrogen ion concentration. It is impossible to generalize on this question with the data at hand. However, certain facts seem to be true. At first sight, it would appear that hydrogen ion

concentration decreases when edema disappears; that is, when diuresis occurs. But in almost every instance general clinical improvement took place, coincident with the diuresis, so that these two factors cannot be separated; and consequently it cannot be said that diuresis occurred because hydrogen ion concentration was falling, or *vice versa*. On the other hand, the facts would seem to show that the hydrogen ion concen-

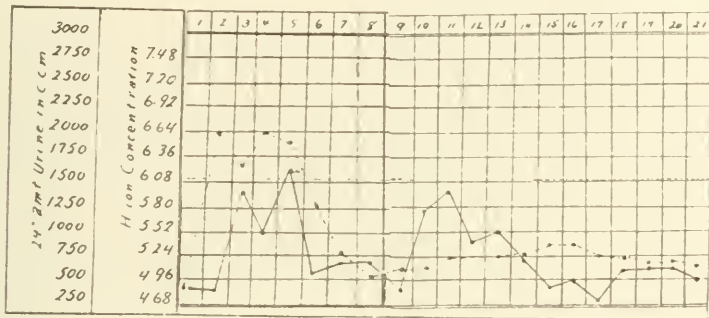


Chart 11.—Case 12. Mitral and aortic disease. Ascites; edema. Made marked improvement for first few days, then improvement ceased and the patient gradually failed to death.

tration depended more on the *severity* of the decompensation than on the *amount* of edema. For in Cases 2 and 8, in which the edema was very slight but in which the patients were very sick at entrance, the initial hydrogen ion concentration was as great as in persons who had massive edema (Cases 1, 4, 6, 10).

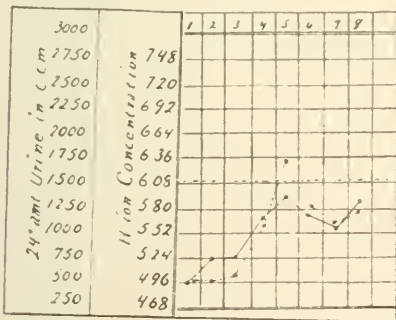


Chart 12.

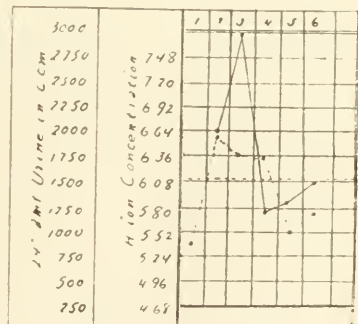


Chart 13.

Chart 12.—Case 14. Arteriosclerosis. Myocardial weakness; small ascites; moderate edema of legs. Made a slow but satisfactory recovery.

Chart 13.—Case 15. Mitral disease and break in compensation. Compensation restored in a week.

Furthermore, in Cases 4 and 6, in which a general temporary improvement occurred at the time of diuresis, with disappearance of edema and in which a stationary period then followed in which there was no edema, but compensation was only partly restored, it will be noted



that the hydrogen ion curve returned to its initial high level after the short period of temporary improvement and remained there even though the patients in the meantime had lost most of their edema.

In one case, No. 11, there were two periods of diuresis. During the first period there was no general improvement, and the curve for hydrogen ion concentration remained level. The second diuretic period was attended by marked clinical improvement of a few days' duration, and this time the hydrogen ion concentration decreased markedly.

#### CONCLUSIONS

1. The hydrogen ion concentration of the urine from individuals with severe cardiac decompensation is higher than the normal.
2. The hydrogen ion concentration follows the general clinical course, becoming normal when compensation is restored.
3. It could not be shown that there was any definite relation between the hydrogen ion concentration of the urine and edema in the cases studied.

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## CLINICAL STUDIES ON ACID BASE EQUILIBRIUM AND THE NATURE OF ACIDOSIS \*

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It is the purpose of this paper to present experimental evidence in favor of the view that varying grades of acidosis may exist, apart from diabetes and case in which beta-oxybutyric acid is produced, in many pathological conditions not previously suspected.

In health and in most diseases the very slightly alkaline reaction of the blood and body tissues is now known to remain practically constant.<sup>1</sup>

Walter,<sup>2</sup> long ago, showed that when an animal is killed by injecting an acid into the blood-stream, the blood apparently still remains alkaline. In disease, slight variations of reaction may occur, but death soon ensues if the change, as determined by the most accurate measurements, is appreciable.<sup>3</sup> In human pathology any change is always toward acidity. Such are the main facts regarding the variation of reaction of the blood.

In the body there is a constant production of acid substances as excretory products of metabolism. These acid bodies combine with base in the blood and are transported to the lungs and kidneys for excretion. Carbonic acid is excreted by the lungs. It devolves mainly on the kidney to excrete sulphuric acid, phosphoric acid, acetoacetic acid, beta-oxybutyric acid, etc.<sup>4</sup> If acid be produced more rapidly than it is excreted, or if the mechanism which regulates the excretion be at fault, the supply of base in the body being limited, a disturbance of the normal neutrality equilibrium may reasonably be expected to result.

The one important result of excessive production of acid, which has come to light, is the withdrawal of base from the body, with the attendant depletion of bicarbonate from the blood. The body is not known ever to produce base except ammonia to neutralize such acid. In the acidosis of diabetes the important factor is the excessive production of beta-oxybutyric acid. It is reasonable to suppose that there may be other conditions in which varying degrees of acid in excess of the normal in the

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\* Submitted for publication May 1, 1913.

1. Henderson, L. J.: The Theory of Neutrality Regulation in the Animal Organism. *Am. Jour. Physiol.*, 1908, xxi, 427.

2. Walter, Frederick: Untersuchungen über die Wirkung der Säuren auf den thierischen Organismus, *Arch. f. exper. Pathol.*, 1877, vii, 148.

3. The investigations of H. Benedict show that the concentration of hydrogen ions in diabetic coma is about  $1.5 \times 10^{-7}$ .

4. Henderson, L. J.: A Critical Study of the Process of Acid Excretion. *Jour. Biol. Chem.*, 1911, ix, 403.

body fluids and tissues may exist, due to a disturbance in metabolism or insufficiency of the excretory mechanism. We have in truth succeeded in obtaining experimental evidence to support us in this view and the recent work of Sellards<sup>5</sup> has already revealed such cases. In this paper we are not concerned with particular acid substances or the cause for their existence, but only with the probability that a condition which may properly be regarded as acidosis in the broadest sense does frequently occur in unexpected circumstances.

Apart from diabetes, and cases in which beta-oxybutyric acid is formed, there are many conditions which clinically are frequently designated as "acid intoxications" or "acidoses." In the acute infectious diseases with high temperatures and severe toxemias, "acidity" is not infrequently mentioned. The acid factor in uremic conditions has long been suspected and work has been done to confirm this view.<sup>6</sup>

The scientific investigation of this subject has been considerable. In addition to the chemical analysis of the blood in conditions of acidosis, various means of determining changes in blood reaction have been employed. By means of the concentration cell measurements of the hydrogen ion concentration of blood, determinations of the tension of carbonic acid in the blood, as well as in the alveolar air of the lungs, titrations of the blood with acid or alkali to various indicator end-points, have been made in large numbers. Each of these methods of investigation possesses its peculiar utility, and on the whole such studies tend to support current ideas regarding acidosis.

The fact that in diabetes large quantities of alkali are necessary to make the urine alkaline has long been known. In acute rheumatic fever the same phenomenon is familiar to every practitioner. Sellards<sup>5</sup> has recently called attention to the fact that large amounts of sodium bicarbonate are necessary to influence the acidity of the urine in the uremia of Asiatic cholera and still later<sup>7</sup> in nephritis. Sellards also finds that the urine of normal individuals is rendered alkaline by the ingestion of sodium bicarbonate in smaller quantities than in the case of individuals with nephritis. In the nephritics he calls this phenomenon "increased alkali tolerance," but concludes that it may indicate a condition of acidosis. That large amounts of alkali are required to reduce the acidity of the urine in nephritis has also been observed by v. Hoesslin,<sup>8</sup> who

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5. Sellards, A. W.: Tolerance for Alkalies in Asiatic Cholera. *Phillipine Jour. Sc.*, 1910, v, 313.

6. Straub, H., and Schlayer: Die Urämie eine Säurevergiftung. München. *Med. Wehnschr.*, 1912, lix, 569.

7. Sellards, A. W.: The Determination of Equilibrium in the Human Body between Acids and Bases with Especial Reference to Acidosis and Nephropathies. *Bull. Johns Hopkins Hosp.*, 1912, xxiii, 289.

8. Hoesslin, V.: Ueber die Abhängigkeit der Albuminurie vom Säuregrad des Urin und über den Einfluss der Alkalizufuhr auf Azidität, Albuminurie, Diurese und Chloridausscheidung sowie auf das Harnammoniak. *Deutsch. Arch. f. klin. Med.*, 1912, cv, 147.

recommends alkali as a therapeutic measure in these cases. Recently Martin Fischer's<sup>9</sup> views on the cause and treatment of nephritis have attracted much attention. Mainly on theoretical consideration he advises the use of alkali in nephritis.

For more than a year past in our work on the acidity (hydrogen ion concentration) of the urine in nephritis we have found, as did Sellards and v. Hoesslin, that the ingestion of large quantities of alkali was frequently necessary before the urinary acidity could be diminished.<sup>10</sup>

In our work the effect of alkali in normal and a great variety of pathological cases has been studied by exact but simple quantitative methods. Throughout the entire investigation the method used for determining the acidity of the urine was the one described by us in a previous paper.<sup>11</sup>

For the convenience of clinicians the following modification is suggested:

Reagents:

1. N/10 Disodium phosphate.
2. N/10 Monopotassium phosphate.
3. N/5 Sodium acetate.
4. N/5 Acetic acid.
5. Two per cent. aqueous solution of sodium alizarin sulphonate.
6. Two per cent aqueous solution neutral red.
7. One per cent. alcoholic solution phenolphthalein.
8. Toluol.

Apparatus:

250 c.c. flasks (3286) obtained of Eimer and Amend, New York, have been found satisfactory. Medium sized test tubes of good, clear glass.

The disodium phosphate,  $\text{Na}_2\text{HPO}_4 + 12\text{H}_2\text{O}$ , is exposed to the air for a week whereby it loses 10 molecules of water of crystallization becoming  $\text{Na}_2\text{HPO}_4 + 2\text{H}_2\text{O}$  which is stable. 17.8 grams to one liter makes a N/10 solution; 13.6 grams of  $\text{KH}_2\text{PO}_4$  to one liter makes a N/10 solution; 27.2 grams of  $\text{CH}_3\text{COONa}$  to one liter makes a N/10 solution; N/10  $\text{CH}_3\text{COOH}$  is made in the usual way by titration against a known alkali solution.

Standard solutions of known hydrogen ion concentration ( $\text{H}^+$ ) are made as follows:

No.						$\text{H}^+$
1	0.5' c.c. N/10 $\text{KH}_2\text{PO}_4$	+	240.0 c.c. N/10 $\text{Na}_2\text{HPO}_4$	made up to 500 c.c. with $\text{H}_2\text{O}$		8.7*
2	0.5 c.c. N/10 $\text{KH}_2\text{PO}_4$	+	60.0 c.c. N/10 $\text{Na}_2\text{HPO}_4$	made up to 500 c.c. with $\text{H}_2\text{O}$		8.0*
3	5.0 c.c. N/10 $\text{KH}_2\text{PO}_4$	+	25.0 c.c. N/10 $\text{Na}_2\text{HPO}_4$	made up to 500 c.c. with $\text{H}_2\text{O}$		7.4†
4	5.0 c.c. N/10 $\text{KH}_2\text{PO}_4$	+	11.5 c.c. N/10 $\text{Na}_2\text{HPO}_4$	made up to 500 c.c. with $\text{H}_2\text{O}$		7.0†
5	2.25 c.c. N/5 $\text{CH}_3\text{COOH}$	+	230.0 c.c. N/5 $\text{CH}_3\text{COONa}$	made up to 500 c.c. with $\text{H}_2\text{O}$		6.7‡
6	5.75 c.c. N/5 $\text{CH}_3\text{COOH}$	+	230.0 c.c. N/5 $\text{CH}_3\text{COONa}$	made up to 500 c.c. with $\text{H}_2\text{O}$		6.3
7	11.50 c.c. N/5 $\text{CH}_3\text{COOH}$	+	230.0 c.c. N/5 $\text{CH}_3\text{COONa}$	made up to 500 c.c. with $\text{H}_2\text{O}$		6.0
8	23.00 c.c. N/5 $\text{CH}_3\text{COOH}$	+	230.0 c.c. N/5 $\text{CH}_3\text{COONa}$	made up to 500 c.c. with $\text{H}_2\text{O}$		5.7
9	57.5 c.c. N/5 $\text{CH}_3\text{COOH}$	+	230.0 c.c. N/5 $\text{CH}_3\text{COONa}$	made up to 500 c.c. with $\text{H}_2\text{O}$		5.3
10	115.00 c.c. N/5 $\text{CH}_3\text{COOH}$	+	230.0 c.c. N/5 $\text{CH}_3\text{COONa}$	made up to 500 c.c. with $\text{H}_2\text{O}$		5.0
11	230.00 c.c. + 230.0 c.c. N/5 $\text{CH}_3\text{COOH}$		N/5 $\text{CH}_3\text{COONa}$	made up to 500 c.c. with $\text{H}_2\text{O}$		4.7

\* Phenolphthalein.

† Neutral red and sodium alizarin sulphonate.

‡ This and remainder of table sodium alizarin sulphonate.

9. Fischer, Martin, H.: Nephritis, 1912, John Wiley and Sons, N. Y.

10. Our work was carried on for some time without knowledge of the work of Sellards.

11. Henderson, L. J., and Palmer, W. W.: Intensity of Urinary Acidity in Normal and Pathological Conditions. Jour. Biol. Chem., 1913, xiii. 393.



TABLE 1.—RESPONSE OF THE KIDNEY TO FOUR GRAMS OF SODIUM BICARBONATE, NORMAL CASES.

No.	Time Alkali Given	+ H Before	1		2		3		4		5		6		7		Diagnosis
			Time	+ H	Time	+ H	Time	+ H	Time	+ H	Time	+ H	Time	+ H			
1	10.00	7.40	11.00	8.30	12.00	7.48	1.00	7.48	2.00	7.40	3.00	5.85	.....	.....	.....	.....	Normal.
2	10.00	5.85	11.00	8.30	12.00	8.15	1.00	6.85	2.00	6.00	3.00	7.20	12.00	*	.....	7.48	Normal.
3	10.00	5.60	11.00	7.48	12.00	7.88	1.00	7.00	2.00	7.48	3.00	7.40	.....	*	.....	7.40	Normal.
4	10.00	5.15	11.00	7.20	12.00	7.20	1.00	6.70	2.00	7.00	3.00	7.20	.....	*	.....	5.70	Normal.
5	10.00	6.30	11.00	6.85	12.00	7.48	1.00	6.85	2.00	5.30	.....	.....	.....	.....	.....	.....	Normal.
6	10.00	6.00	11.30	7.20	.....	.....	.....	.....	2.00	7.48	.....	4.00	7.40	.....	.....	.....	Normal.

\* Next a. m.

TABLE 2.—RESPONSE OF THE KIDNEY TO FOUR GRAMS OF SODIUM BICARBONATE. PATHOLOGICAL CASES. HOURLY OBSERVATIONS

No.	Time Alkali Given	H <sup>+</sup> Before Alkali	1		2		3		4		5		6		7		Diagnosis
			Time	+ H	Time	+ H	Time	+ H	Time	+ H	Time	+ H	Time	+ H	Time	+ H	
1	10.00	5.40	11.00	7.48	12.00	7.20	1.00	6.50	2.00	5.70	3.00	5.70	12.00	5.70	*	5.70	Arteriosclerosis
2	11.00	5.70	12.20	7.20	1.05	5.85	2.20	7.00	6.30	7.48	.....	.....	.....	.....	*	7.48	Weak heart. Chronic neph.
3	10.00	5.00	11.00	5.15	12.00	7.20	1.00	7.20	.....	.....	.....	.....	.....	.....	.....	.....	Hypertension. Phthisis. Pneumothorax.
4	10.00	7.48	11.00	8.15	12.00	8.30	1.00	8.50	2.00	8.15	3.00	8.15	.....	.....	.....	.....	Incipient pul. tuberculosis.
5	10.15	5.30	11.05	6.15	12.00	6.70	1.15	5.70	2.15	6.50	3.15	6.70	11.00	6.00	*	6.15	Pernicious anemia.
6	10.00	5.30	11.00	7.20	12.00	7.20	1.00	5.85	.....	.....	.....	.....	.....	.....	.....	.....	Gastric cancer.
7	10.00	5.60	11.20	7.48	1.00	7.40	3.00	7.20	8.00	7.48	.....	.....	.....	.....	.....	.....	Acute tonsillar infection.
8	10.00	7.20	10.40	8.00	11.55	8.30	1.10	8.00	.....	.....	.....	.....	.....	.....	.....	.....	Acute endocarditis. Severe anemia.

\* Next a. m.

TABLE 3.—RESPONSE OF THE KIDNEY TO FOUR GRAMS OF SODIUM BICARBONATE. PATHOLOGICAL CASES. TWO-HOURLY OBSERVATIONS\*

No.	Time Alkali Given	H Before Alkali	1		2		3		4		5		7		Diagnosis
			Time	+ H	Time	+ H	Time	+ H	Time	+ H	Time	+ H	Time	+ H	
1	11.00	5.70	1.00	7.00	3.00	7.48	.....	.....	.....	7.20	.....	.....	.....	.....	Acute nephritis.
2	9.00	5.00	11.00	7.40	1.00	7.40	3.00	7.40	6.30	7.20	.....	.....	.....	6.70	Acute nephritis.
3	9.00	7.20	11.00	7.48	1.00	7.48	3.00	8.15	.....	.....	.....	.....	.....	7.48	Acute nephritis.
4	9.30	4.70	11.00	6.70	1.30	6.70	3.30	5.70	6.20	6.85	.....	.....	.....	5.40	Acute nephritis.
5	1.00	5.40	3.00	7.48	5.00	7.48	6.00	7.40	.....	.....	.....	.....	.....	7.00	Acute appendicitis.
6	10.00	6.30	12.00	7.48	1.00	7.48	2.00	7.40	3.00	7.40	.....	.....	.....	7.40	Chronic glomerulonephritis, hypertension.
7	10.00	5.85	12.00	6.50	1.00	6.00	3.00	6.30	.....	.....	.....	.....	.....	.....	Secondary anemia, unexplained
8	9.00	5.30	11.00	7.00	1.00	7.00	3.00	7.20	.....	.....	.....	.....	.....	.....	Pleurisy with effusion.
9	10.30	5.70	12.30	7.20	2.30	7.48	3.30	7.48	.....	.....	.....	.....	.....	6.30	Empyema following lobar pneumonia.
10	9.00	4.85	11.00	5.40	1.00	6.70	3.00	5.70	.....	.....	.....	.....	.....	.....	Cardiac decompensation, aortic and mitral disease.
11	11.00	4.85	1.00	7.20	2.00	7.20	3.00	8.70	.....	.....	.....	.....	.....	.....	Cardiac decompensation.
12	9.00	6.00	9.00	7.20	11.00	7.20	1.30	8.15	3.50	8.30	6.00	7.20	.....	5.50	Mitral disease.
13	9.30	6.50	11.00	7.40	1.00	7.40	.....	.....	.....	.....	.....	.....	.....	.....	Mitral disease.
14	9.00	5.50	11.00	8.15	1.00	7.48	3.00	7.00	.....	.....	.....	.....	.....	.....	Acute bronchitis.
15	9.00	5.15	11.00	6.00	1.00	7.00	3.00	4.70	.....	.....	.....	.....	.....	5.00	Acute bronchitis.
16	1.30	6.00	3.15	7.20	5.30	7.48	.....	.....	.....	.....	.....	.....	.....	.....	Influenza, bronchopneumonia
17	10.00	6.00	12.00	7.20	3.00	7.20	4.30	7.20	.....	.....	.....	.....	.....	.....	Hysteria.
									.....	.....	.....	.....	.....	.....	Renal calculus.

\* No test given on 6th.

† Next a. m.

TABLE 4.—RESPONSE OF THE KIDNEY TO FOUR GRAMS OF SODIUM BICARBONATE. SPECIMENS COLLECTED AT IRREGULAR INTERVALS\*

No.	Time. Alkali Given	1		2		3		4		7		Diagnosis
		Time	†	Time	†	Time	†	Time	†	Time	†	
1	8.00	11.45	8.00	3.10	7.48	5.00	7.48	.....	.....	.....	.....	Normal.
2	11.00	4.00	7.00	5.30	5.50	6.30	5.50	.....	.....	.....	.....	Normal.
3	10.00	12.45	6.30	4.30	5.85	.....	.....	.....	.....	.....	.....	Arteriosclerosis with weak heart.
4	7.00	8.00	5.85	10.30	7.40	12.55	7.00	3.50	7.40	.....	.....	Acute infection.
5	10.00	5.70	7.40	3.00	7.40	5.30	5.40	.....	.....	.....	.....	Acute plumbism.
6	9.00	5.50	11.00	6.00	.....	.....	.....	.....	.....	.....	.....	Carcinoma pancreas; metastasis in liver.
7	11.30	6.00	5.30	7.48	.....	.....	.....	.....	.....	.....	.....	Acute rheumatic fever.
8	8.00	8.50	7.00	1.30	6.70	2.30	7.00	.....	.....	.....	.....	Carcinoma of bladder.
9	9.30	11.30	7.40	3.30	7.40	5.00	7.40	.....	.....	.....	.....	Gastric neurosis.
10	12.00	5.30	1.45	8.00	.....	.....	.....	.....	.....	.....	.....	Gastric neurosis.
11	10.15	7.00	5.00	7.48	6.70	12.00	5.60	.....	.....	.....	.....	Duodenal ulcer.
12	9.00	7.00	11.00	8.00	.....	.....	.....	.....	.....	.....	.....	Amebic dysentery.
13	9.30	6.50	10.30	7.40	3.40	6.30	7.48	.....	.....	.....	.....	Typhoid fever.
14	11.00	5.85	12.30	6.30	6.00	6.30	5.70	9.00	5.70	.....	.....	Typhoid fever.
15	8.50	5.85	3.30	8.15	5.00	8.00	.....	.....	.....	.....	.....	Gastric ulcer.
16	7.30	5.70	10.15	7.00	1.30	7.48	7.30	.....	.....	.....	.....	Hyperacidity.
17	11.00	5.85	3.00	6.00	8.00	4.20	.....	.....	.....	.....	.....	Lung abscess.
18	1.10	5.30	3.45	6.45	7.40	.....	.....	.....	.....	.....	.....	Lung abscess.

\* No tests given on fifth and sixth.

† Next a. m.



These standard solutions are put up in suitable bottles and a few cubic centimeters of toluol poured over each to prevent the growth of yeasts and molds.

In each of nine flasks is placed a 10 c.c. sample of each of the standard solutions, 3 to 11, the volume is made up to 250 c.c. with distilled water and 5 drops of the alizarin added. Care is necessary to have the concentration of indicator exactly equal in all cases. Ten c.c. of urine are next introduced into another flask and distilled water and indicator are added. The color of the diluted urine solution is next matched with one of the standard series.

If the reaction as thus measured falls between Solutions 3 and 5 a similar comparison is made using neutral red (5 drops) as an indicator. If the reaction is more alkaline than Solution 3, undiluted urine is matched in test-tubes against undiluted standard Solutions 1 and 2 using phenolphthalein (10 drops) as indicator. In case the reaction falls between the standard solutions, rough interpolation is made. The standard series of flasks containing the alizarin will keep, if corked, for three to four days in cool weather; in warm weather they should be made up fresh every other day. The solutions containing neutral red and phenolphthalein are made every time their use is required.

A word of explanation concerning the values thus determined is necessary. In physical chemistry acidity and alkalinity are expressed in terms of hydrogen or hydroxyl ion concentration. Pure water,  $\text{H}_2\text{O}$ , ionizes; that is, a few molecules break up into  $\text{H}^+$  and  $\text{OH}^-$  ions in equal numbers, and we have neutrality. The amount of ionized hydrogen in water is exceedingly small, about 1 gram in 10,000,000 liters at 25 C. The phrase "hydrogen ion concentration" signifies the quantity of hydrogen ions present, expressed in terms of normality. Hence in water containing 1 gram of ionized hydrogen in 10,000,000 liters, or 0.000,000, 1 gram in one liter, the hydrogen ion concentration would be 0.000,000, 1 N, which may be written  $1/10,000,000$  N, or expressed more conveniently algebraically,  $1 \times 10^{-7}$  N. We have then for the definition of neutrality, acidity and alkalinity —

$(\text{H}^+) = 1 \times 10^{-7} = (\text{OH}^-)$  neutrality.

$(\text{H}^+) > 1 \times 10^{-7} > (\text{OH}^-)$  acidity.

$(\text{H}^+) < 1 \times 10^{-7} < (\text{OH}^-)$  alkalinity.

$(\text{H}^+)$  stands for hydrogen ion concentration.

$(\text{OH}^-)$  stands for hydroxyl ion concentration.

In normal blood,  $(\text{H}^+) = 0.4 \times 10^{-7}$ , which is approximately the reaction of Solution 3.

In our work we have adopted the logarithmic notation. All logarithmics are of course negative, and for convenience the minus signs are omitted. The following table presents the conversion of the logarithmic

notation into actual concentration of ionized hydrogen. It will be seen that the smaller the logarithms the greater acidity.<sup>12</sup>

Log.	$\frac{+}{H}$	Log.	$\frac{+}{H}$
4.6	$250 \times 10^{-7}$	6.4 *	$4.0 \times 10^{-7}$
4.8	$160 \times 10^{-7}$	6.6	$2.5 \times 10^{-7}$
5.0	$100 \times 10^{-7}$	6.8	$1.6 \times 10^{-7}$
5.2	$63 \times 10^{-7}$	7.0	$1.0 \times 10^{-7}$
5.4	$40 \times 10^{-7}$	7.2	$0.63 \times 10^{-7}$
5.6	$25 \times 10^{-7}$	7.4	$0.40 \times 10^{-7}$
5.8	$16 \times 10^{-7}$	7.6	$0.25 \times 10^{-7}$
6.0	$10 \times 10^{-7}$	7.8	$0.16 \times 10^{-7}$
6.2	$6.3 \times 10^{-7}$	8.0	$0.10 \times 10^{-7}$

This method of studying urinary acidity is exceedingly simple, permitting numerous and accurate observations, and is well adapted to the range of reaction which may be encountered. The routine carried out in every case was the administration by mouth of 4 to 8 grams of sodium bicarbonate, at which time a specimen of urine was taken, as well as an hourly or two hourly specimen for several hours thereafter, the acidity being measured in each specimen. The acidity (hydrogen ion concentration) is recorded as the logarithm of the actual value. Hence, the smaller the logarithm the greater the acidity. (See note above.)

In suitable cases in which there was no response of urinary acidity to a normal dose of 4 grams of sodium bicarbonate, a sufficient quantity of the alkali was given to reduce the acidity of the urine. As soon as the effect of the alkali was apparent the sodium bicarbonate was omitted, the urine allowed to regain its former acidity and a second test was made with 4 grams of sodium bicarbonate. These observations were made in cases which showed very little clinical change during the course of the experiment.

As may be seen on examination of Tables 1, 2, 3, 4 and 5, the effect of small amounts of sodium bicarbonate is shown normally in the urine by a prompt and marked reduction of the acidity. This effect manifests itself soon after alkali intake. When hourly observations were made, in both normal and pathological cases, marked reduction of the acidity occurs in about an hour, the maximum effect in a little over one hour. In the cases when two hourly observations were made, marked reduction of urinary acidity was noted at the first observation after the alkali was taken. The average change in cases when there was a normal response to alkali was 1.6%, which amounts approximately to a fifty-fold reduction in urinary acidity. After alkali intake the average degree of alkalinity reached was 7.60, which is slightly below the reaction of blood.

12. For detailed discussion of this subject see: Neubauer-Huppert: *Analyse des Harns.*, 1910, i. 1. Neuberg. Carl: *Der Harn*, 1911, ii, 1396.

TABLE 5.—RESPONSE OF THE KIDNEY TO EIGHT GRAMS OF SODIUM BICARBONATE

No.	Time Alkali Given	H before Alkali	1		2		3		4		5		6		Diagnosis
			Time	† H	Time	† H	Time	† H	Time	† H	Time	† H	Time	† H	
1	10.00	5.70	11.00	8.50	12.00	8.30	1.00	6.50	2.00	6.50	3.00	7.40	.....	.....	Normal.
2	10.00	7.40	11.00	8.50	12.00	8.70	1.00	8.50	2.00	8.50	3.00	8.50	.....	.....	Normal.
3	10.30	6.70	6.30	8.50	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Normal.
4	10.00	5.85	1.00	8.70	2.00	8.70	3.00	.....	.....	.....	.....	.....	.....	.....	Normal.
5	10.00	6.70	11.00	7.48	12.00	8.70	1.00	8.50	2.00	8.70	5.00	8.50	.....	.....	Normal.
6	10.15	5.85	11.30	8.70	12.30	8.70	1.30	7.48	3.00	7.40	.....	.....	.....	.....	Chronic glomerulonephritis.
7	10.00	5.70	11.00	5.60	12.15	6.70	1.15	7.40	3.15	7.40	6.00	5.70	*	5.30	Hemophilia.
8	10.00	7.40	11.00	7.48	12.00	8.15	1.00	7.48	3.00	7.48	7.15	7.48	*	7.40	Acute tonsillitis.
9	10.00	5.60	11.00	7.40	12.00	7.20	1.00	7.00	2.00	7.00	3.00	7.20	*	5.85	Acute rheumatic fever.
10	10.00	7.40	11.00	7.48	12.15	8.15	1.15	8.00	3.15	7.48	9.00	6.30	*	7.40	Chronic glomerulonephritis; hypertension.
11	10.00	5.40	11.00	6.00	12.00	6.30	1.00	6.00	2.00	5.30	3.00	5.30	*	7.20	Cardiorenal disease.
12	3.30†	6.00	5.15	8.30	6.15	8.70	.....	.....	.....	.....	.....	.....	.....	.....	Cardiac decompensation; mitral disease.

\* Next morning. † P. M.

In a few cases there was a delay of four to six hours before the maximum reduction in urinary acidity was reached. Because of the small number and variety of diseases in which this delay was observed, we make no attempt to explain it.

A great variety of diseases (Table 6) showed in certain cases no response to 4 grams of sodium bicarbonate. This lack of response to alkali occurred most frequently in patients with kidney disease. In comparing Table 6 with Tables 1, 2, 3, 4 and 5, it would appear, however, that the response or lack of response to alkali is not confined to any particular disease or group of diseases, but is more or less an individual phenomenon.

Several of the cases reported in Table 6 which showed no response on the initial 4 grams of sodium bicarbonate required varying amounts of alkali, 12 to 112 grams, before any effect on the acidity of the urine could be detected. With two exceptions after the urine had been allowed to regain its former acidity, 4 grams of sodium bicarbonate produced a prompt and marked effect on the urinary acidity, as it did in Tables 1, 2, 3, 4 and 5. This phenomenon we believe to be due to a need on the part of the body for alkali—to acidosis, in short. In kidney disease the prompt response to the second test made with the alkali would indicate that the kidney lesion *per se* was not a factor in the lack of response in the first instance. From unpublished data there seems to be no relation between the degree of acidosis and ammonia excretion in cases in which beta-oxylutyric acid is not produced.

All these observations lead us to the belief that the one well marked effect of over production of acid — decrease of sodium bicarbonate in the blood — is a frequent occurrence in many and various pathological conditions. As a result of our exact studies of urinary reaction and its change after the administration of alkali, together with what appears to us as the crucial experiment of the second administration of alkali immediately after the urine has become once more acid, we agree in the main with Sellards' conclusions, and believe that we have provided a more secure foundation for them. However, we regard his term "tolerance" for alkali, in the cases which do not respond to the administration of alkali by a diminution of urinary acidity, as unfortunate and misleading. We are strongly disposed to the view, which cannot be regarded as fully proved, that the phenomenon is merely due to retention of a normal constituent of the organism until its concentration — lowered by pathological processes — has been restored.

With these observations and considerations in mind we suggest that a condition of acidosis may be assumed to exist when the administration of a quantity of alkali equivalent to one liter of tenth normal solution fails to produce a diminution in the acidity of the urine. Hence the



TABLE 6.—No RESPONSE OF THE KIDNEY TO FOUR GRAMS OF SODIUM BICARBONATE\*

No.	Time Alkali Given	+ H Before Alkali	1		2		3		4		5		6		7		Diagnosis
			Time	+ H	Time	+ H	Time	+ H	Time	+ H	Time	+ H	Time	+ H	Time	+ H	
1	11.00	5.60	2.15	5.85	3.00	6.00	11.45	5.15	.....	.....	.....	.....	.....	.....	.....	.....	Pleurisy with effusion.
2	10.00	5.00	1.00	5.30	3.00	5.30	5.30	5.40	.....	.....	.....	.....	.....	.....	.....	.....	Pleurisy with effusion.
3	11.00	5.30	3.25	5.40	6.00	5.40	11.30	5.50	.....	.....	.....	.....	.....	.....	.....	.....	Pleurisy with effusion.
4	11.00	6.15	12.00	5.85	1.00	5.70	2.00	5.60	3.00	5.60	4.00	5.70	6.30	6.15	.....	.....	Diabetes mellitus
5	11.00	5.30	12.00	5.40	1.00	5.15	2.00	5.30	3.00	5.30	4.00	5.40	12.10	5.70	.....	.....	Chronic glomerulonephritis.
6	10.00	4.85	2.00	4.85	4.00	4.85	12.00	4.85	.....	.....	.....	.....	.....	.....	.....	.....	Chronic glomerulonephritis.
7	10.00	5.30	11.00	5.30	12.00	5.60	3.00	5.30	.....	.....	.....	.....	.....	.....	†	5.50	Chronic glomerulonephritis.
8	12.30	5.00	5.00	5.00	7.15	4.85	10.50	5.15	.....	.....	.....	.....	.....	.....	†	4.85	Chronic nephritis
9	10.00	5.30	11.00	5.15	3.00	5.15	8.00	5.30	.....	.....	.....	.....	.....	.....	†	5.15	Arteriosclerosis; weak heart.
10	11.00	5.40	1.00	5.40	11.30	5.30	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Arteriosclerosis; weak heart.
11	10.00	4.70	11.00	4.70	12.00	4.70	1.00	4.70	2.00	4.70	3.00	4.70	.....	.....	.....	.....	Hypernephroma.



TABLE 7.—TABLE OF AVERAGES

Number of Table	Average H + Before Alkali	Maximum H + Before Alkali	Minimum H + Before Alkali	Average Time Before Effect; Hrs.	Average Time of Maximum Effect; Hrs.	Average Change H +	Maximum H + Change	Minimum H + Change	Average Low + Limit H	Low Limit H + After Alkali	High Limit H + After Alkali	
1	6.05	5.15	7.40	1.1	1.2	1.65	2.45	0.90	7.70	8.30	7.20	Normal cases; hourly observations; 4 grams of alkali.
2	5.87	5.30	7.48	1.1	1.6	1.65	2.20	1.02	7.52	8.50	7.20	Pathological cases; hourly observations; 4 grams of alkali.
3	5.65	4.70	7.20	2.0	3.5	1.80	3.85	0.65	7.45	8.70	6.50	Pathological cases; two-hourly observations; 4 grams of alkali.
4	5.83	4.85	* 7.00	.....	.....	1.50	2.70	0.48	7.25	8.15	6.00	Pathological cases; irregular observations; 4 grams of alkali.
5	6.56	5.40	7.48	.....	.....	1.84	2.85	0.67	8.14	8.70	6.30	Normal and pathological cases; 8 grams of alkali.
6	5.30	4.70	6.30	.....	.....	.....	.....	.....	.....	.....	.....	Pathological cases showing no response to 4 grams of alkali.
...	*	.....	.....	1.4	2.1	1.67*	2.80	0.75	7.60*	8.30	6.63	General average in cases showing a response to alkali.

\* Average obtained from all cases reported in previous tables.

condition of acidosis, so long vague and ill-defined, may at length be considered, whatever its origin and variety, as a depletion of bicarbonate from the blood, which would naturally reveal itself to the above test.<sup>13</sup>

We believe that these experiments also establish absolute indications for or against the therapeutic use of alkalies in many conditions. With a disturbance in the acid-base equilibrium in the body, which is probably due to an alkali depletion, it seems to us reasonable to attempt to restore the normal equilibrium by the administration of alkali. Certain facts concerning the use of alkali as a therapeutic measure should, however, be recognized. Alkali may be considered in the same category as water, good food, fresh air, and good nursing. As the result of many observations on the acidity of the urine in normal and pathological cases<sup>11</sup> we have found no case, without experimental interference, to have an acidity less than blood. In determining the extreme variations in acidity of the urine in normal and pathological individuals<sup>14</sup> we find that alkali given in amounts sufficient to push the reaction of the urine below that of blood a well marked albuminuria not infrequently results.

The gastro-intestinal disturbances from the use of too much alkali are well known. On the other hand there is no doubt that in many pathological conditions alkali in proper amounts makes the patients feel better. The choice of alkali depends on the individuals' taste and tolerance as well as the physicians' preference. Alkali should be given in large amounts (if sodium bicarbonate, 4 grams, three or four times a day) until the effect on the urinary acidity is noted, and thereafter in sufficient amounts to keep the reaction of the urine slightly more acid than blood.<sup>15</sup> The amount to accomplish this is frequently very small, not more than 4 grams daily. The same indications pertain to the use of alkali as a diuretic. In all of our cases with or without edema when there was a diuresis following the administration of alkali, the diuresis did not occur until the reduction of the acidity of the urine was established. It should be stated, however, that in some cases we have seen marked diuresis following the use of alkali; in others no diuresis.

When the therapeutic use of alkali is considered we recommend that at least the effect on the reaction of the urine be carefully watched or that the following procedure be followed:

1. Sodium bicarbonate, 4 grams, preferably between meals.

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13. In all investigations on this subject it appears to be necessary to estimate with moderate accuracy the hydrogen ion concentration of the urine.

14. Henderson, L. J., and Palmer, W. W.: Extreme Variations of the Concentration of Ionized Hydrogen in Human Urine. *Jour. Biol. Chem.*, 1913, xiv, 81.

15. We have found it convenient to estimate the amount of alkali given in terms of a tenth normal solution. Four grams of sodium bicarbonate makes approximately 500 c.c. of a tenth normal solution.



TABLE 8.—CASES IN WHICH THERE WAS NO RESPONSE TO FOUR GRAMS OF SODIUM BICARBONATE

Alkali was given until urine became alkaline, then discontinued until the urine had regained its former acidity, when a second test with 4 grams of sodium bicarbonate was made.

Number.		1		2		3		4		5		6		Remarks
		Time	+ H	Time	+ H	Time	+ H	Time	+ H	Time	+ H	Following Morning Time	+ H	
1	Before* After†	12.00 11.00	5.70 7.48	1.00 12.00	5.15 7.48	2.00 1.00	5.15 7.00	..... 2.00	..... 7.00	..... 3.00	..... 7.00	..... .....	..... .....	Pleurisy with effu- sion; 12 gm. alkali required to make urine alkaline.
2	Before After	1.00 12.00	5.40 6.00	11.30 1.40	5.40 6.00	..... 3.30	..... 6.00	..... 4.35	..... 5.70	..... 8.00	..... 5.60	..... .....	..... .....	Arteriosclerosis; 24 gm. alkali required to make urine alkaline.
3	Before After	11.00 11.00	5.30 5.40	12.00 1.00	5.60 6.30	2.00 3.00	5.40 6.30	3.00 .....	5.40 .....	..... .....	..... .....	..... .....	..... .....	Pernicious anemia; 12 gm. alkali req'd to make urine alkaline.
4	Before After	11.00 11.00	5.15 6.30	12.00 1.00	5.15 7.20	1.00 3.00	5.15 5.85	2.00 10.00	5.15 7.48	3.00 1.00	5.15 7.48	..... 5.00	..... 7.48	Acute nephritis. Marked ascites and edema; 112 grams alkali before urine became alkaline.
5	Before After	3.25 8.00	5.40 5.60	6.00 2.00	5.40 7.00	11.00 6.30	5.50 7.00	..... .....	..... .....	..... .....	..... .....	..... 7.00	..... 7.00	Pleurisy with effu- sion; 36 grams alkali before urine became alkaline.
6	Before After	5.00 3.25	5.00 6.30	7.15 9.30	4.85 7.20	10.30 .....	5.15 .....	..... .....	..... .....	..... .....	..... .....	7.00 6.30	4.85 7.20	Chronic nephritis; hypertension; no edema; 40 gm. alkali before urine became alkaline.



2. Determine the acidity on specimens of urine obtained at time of administration of the alkali and on single specimens passed within the following six or eight hours.

3. If marked reduction in urinary acidity occurs, alkali is not indicated and should not be given.

4. If no reduction is noted, alkali should be given until the desired effect on the urine is noted.

#### SUMMARY

1. A condition of acidosis appears to exist in a great variety of pathological conditions.

2. A simple clinical test is proposed for the detection of acidosis, whatever its origin.

3. A rational use and control of alkali in therapeutics is proposed.

## STUDY XX: THE EFFECT OF DIURETIC DRUGS ON THE LIFE OF ANIMALS WITH SEVERE ACUTE NEPHRITIS \*

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In a previous paper, Christian and O'Hare<sup>1</sup> have reported a detrimental effect of the diuretic drug, diuretin, in severe acute nephritis produced in rabbits by injections of uranium nitrate. Therefore, we were interested to study the effects of other drugs commonly employed by clinicians as diuretics, such as theocin, caffeine, potassium acetate and spartein sulphate. The latter drug is included, as it is used by some clinicians as a diuretic, though not generally regarded as a diuretic in the same sense as the others.

Acute nephritis was produced in rabbits by uranium nitrate. Three and one-half mg. of uranium nitrate was dissolved in 1 c.c. of sterile distilled water, and the solution so made was given intravenously. Each animal received two doses twenty-four hours apart of 3.5 mg. of uranium nitrate per kilo of body weight. Uranium nitrate was employed to produce the nephritis because it causes a lesion bearing much resemblance to acute nephritis in man, and because previous work in this laboratory had made us familiar with its action. With these doses the nephritis is of a severe type, usually rapidly fatal.

The diuretic drugs were dissolved in sterile distilled water and were given intravenously, twice daily, the first dose twenty-four hours after the last (second) uranium injection. The drugs were given in amounts in proportion to those ordinarily given to an adult man weighing 170 pounds; that is, theocin<sup>2</sup> was given in 5 mg. doses per kilo of body

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\* This is one of a series of studies on experimental cardio-renal disease. Study I, Smith, *Boston Med. and Surg. Jour.*, 1908, clviii, 696; Study II, Christian, *Boston Med. and Surg. Jour.*, 1908, clx, 8; Study III, Christian, *Jour. Am. Med. Assn.*, 1909, liii, 1792; Studies IV-XV, Christian, Smith and Walker, *THE ARCHIVES INT. MED.*, 1911, viii, 468-551; Study XVI, Christian and O'Hare, *THE ARCHIVES INT. MED.*, 1913, xi, 517; Studies XVII and XVIII, O'Hare, *THE ARCHIVES INT. MED.*, 1913, xii, 49, 61; Study, XIX, Christian and O'Hare, *Jour. Med. Research*, 1913, xxviii, 227.

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\* Submitted for publication May 21, 1913.

1. Christian and O'Hare: Study XVI. *THE ARCHIVES INT. MED.*

2. Theocin sodium acetate (soluble theocin). F. Bayer & Co., Germany: Caffein, Merck & Co.; potassium acetate, obtained of local druggist; spartein sulphate, Merck & Co.



weight, caffein in 3 mg. doses per kilo of body weight, potassium acetate in 14 mg. doses per kilo of body weight, and spartein sulphate in 0.42 mg. doses per kilo of body weight. Because of the slight solubility in water of caffein, all the drugs were dissolved in equal and rather large amounts of water. Each solution was made so that the above dose of the drugs was contained in 4 c.c. of water; therefore, each animal received with the drug 4 c.c. of water per kilo of body weight.

Since such large amounts of water when given intravenously might be detrimental or otherwise in such cases, and secondly, since water itself is a good diuretic, the effect of the water was tested on other rabbits having a similar experimental nephritis. To them was given intravenously, twice daily 4 c.c. of sterile distilled water per kilo of body weight.

Normal rabbits were given water alone in similar amounts by the same methods, and also similar doses of the various drugs dissolved in equal amounts of water in order to determine the toxicity of the drugs. These animals serve as controls for the experiments with acute nephritis.

The effect of the diuretics, whether detrimental or beneficial, was judged by the length of life of the animals receiving them as compared with that of control rabbits which had a similar acute nephritis. The length of life in all cases was reckoned from the first injection of the uranium nitrate until the death of the animal.

#### FIRST SERIES OF EXPERIMENTS

In the first series (Table 1) 33 rabbits of varying weights were given two intravenous injections of uranium nitrate, 3.5 mg. per kilo of body weight, twenty-four hours apart. After an interval of twenty-four hours more a group of 7 of these rabbits were given intravenous injections of theocin 5 mg. (in 4 c.c. of water) per kilo of body weight twice daily; 7 others were given similarly caffein 3 mg. per kilo of body weight; a third group of 7 potassium acetate 14 mg. per kilo of body weight, and a fourth group of 7 were given spartein sulphate 0.42 mg. per kilo of body weight; the remaining five rabbits were used as controls and were given no diuretics, these having had only the uranium nitrate. Of the 7 given theocin, 1 died in  $3\frac{1}{3}$  days, 4 in 4 and a fraction days, 2 in 5 and a fraction days. The average term of life of the group was  $4\frac{4}{7}$  days, and the average number of doses of theocin per animal was seven. The group given potassium acetate ran a parallel course to those given theocin; 1 died in  $3\frac{1}{3}$  days, 3 in 4 and a fraction days, and 3 in 5 and a fraction days. The average length of life of this group was  $4\frac{4}{7}$  days, and the average number of doses six per animal. The group given caffein lived slightly longer than the two previous groups. Three rabbits died in 4 and a fraction days, 3 in 5 days and 1 lived  $6\frac{2}{3}$  days; this latter animal brought the average length of life of the group up to 5 days, and the

TABLE 1.—EFFECT OF DIURETIC DRUGS IN URANIUM NEPHRITIS

[illegible]

average number of doses per animal up to seven. The group of spartein animals lived a longer time both singly and collectively than did the previous three groups. One spartein rabbit lived  $4\frac{1}{3}$  days, 4 lived 5 and a fraction days, 1 lived 10 days, and 1 survived entirely, the injections being discontinued on the seventh day. The average length of life of this group, excluding the surviving rabbit, was 6 and a fraction days, and the average number of doses per animal was eight and two-sevenths. Of the 5 controls, 2 died in 4 and a fraction days, 1 on the 8th day, 1 on the 10th and 1 on the 14th day; an average length of life of  $8\frac{1}{4}$  days.

In comparing the effect of these diuretic drugs on this series of 33 rabbits having an acute nephritis, it is evident that theocin and potassium acetate shortened the average life of the animal by almost one-half, and individually only two controls died before all the theocin and potassium acetate animals died. Caffein shortened the average length of life by 3 days, and all were dead before 3 of the 5 controls; still, caffein was not quite so detrimental as the previous two drugs. It may be said that spartein sulphate also was to some extent detrimental since 5 animals died during the time only 2 controls died; on the other hand, 1 spartein sulphate animal survived while none of the control animals survived. Spartein is much less harmful than the previous drugs used in this series, since only 1 died as early as  $4\frac{1}{3}$  days, and 2 died in  $5\frac{1}{3}$  and  $5\frac{2}{3}$  days, respectively, by which time all animals of the previous groups had died; in addition, 1 spartein rabbit lived 10 days and 1 survived entirely. Therefore, we may conclude that theocin, caffein and potassium acetate as given in these animals are very detrimental in acute experimental nephritis, in that they shorten materially the life of the animal. However, since these drugs were given in rather large amounts of water (4 c.c. per kilo) for intravenous injection, it might be considered that the water played an important part in the acute nephritis fatalities, either by making extra work for the kidneys, or by causing hemolysis of the red blood-cells and consequently hemoglobin poisoning or otherwise. On the other hand, with these large amounts of water, the drugs were greatly diluted and consequently less irritating. In order to determine the ill effects of any of these large amounts of water, the following series of animals were studied.

#### SECOND SERIES OF EXPERIMENTS

The second series of 8 rabbits (Table 2) were given an acute nephritis as in the previous series, and to 4 were given intravenously 4 c.c. of sterile distilled water per kilo of body weight twice daily, the other 4 being controls. Of the 4 given water, 1 died in  $2\frac{1}{3}$  days, 1 in  $3\frac{1}{3}$  days, and 2 died in 4 days, an average length of life of  $5\frac{5}{12}$  days. Of the 4 controls, 1 died in  $3\frac{1}{3}$  days and 3 in 4 days, an average length of life

of 3  $\frac{5}{6}$  days. In comparing the animals individually, it is noted that in each group, 1 died in 3 $\frac{1}{3}$  days, and 2 in 4 days; therefore, each group ran a parallel course for three animals. The fourth animal in the water group died over a day sooner than the fourth control. Consequently one might think that the water played an important part in one animal at least. Since the controls died so shortly after injection and all the water ones as well, we feel that these animals were unusually susceptible to uranium nitrate. That such is frequently the case is well known to those who have used uranium. This is substantiated by 19 control rabbits used in another experiment treated in the same way in which the average length of life was 6 days. Not being satisfied with the unusual early deaths of this series, this part of the work was repeated.

TABLE 2.—EFFECT OF STERILE WATER INJECTIONS IN URANIUM NEPHRITIS

Rabbit No.	Weight	Result	Treatment	C.C. Fluid	No Doses	Animals Dying on Successive Days after First Uranium Injection					
						2 $\frac{1}{3}$ Days	3 $\frac{1}{3}$ Days	4 Days	4 $\frac{2}{3}$ Days	5 $\frac{1}{3}$ Days	Average No. Days
625	1370	died	Ster. H <sub>2</sub> O	5.5	3	....	1	....	....	....	....
626	1370	died	Ster. H <sub>2</sub> O	5.5	4	....	....	1	....	....	3 $\frac{5}{12}$
628	1700	died	Ster. H <sub>2</sub> O	7.	4	*	....	1	....	....	....
627	2070	died	Ster. H <sub>2</sub> O	8.	2	1	....	....	....	....	....
621	1800	died	Controls	....	....	....	....	1	....	....	....
623	2150	died	Controls	....	....	....	....	1	....	....	3 $\frac{5}{6}$
624	1650	died	Controls	....	....	....	....	1	....	....	....
622	1570	died	Controls	....	....	....	1	....	....	....	....

\* This rabbit died immediately following last injection. Exit with convulsions, probably due to embolism.

## THIRD SERIES OF EXPERIMENTS

A third series of 8 rabbits (Table 3) were used to repeat the work of the previous series. Of the 4 rabbits in this series receiving water, 2 died in 4 $\frac{2}{3}$  days and 2 in 5 $\frac{2}{3}$  days, whereas of the 4 controls, 1 died in 4 $\frac{2}{3}$  days and 1 in 5 $\frac{2}{3}$  days, and 2 entirely survived. This substantiates the results of the previous series, in that water in such large amounts intravenously does have in some cases a detrimental effect. But this effect is not constant, as was that with theocin, caffein and potassium acetate; neither was the effect so rapidly fatal since the rabbits receiving water all died in 4 $\frac{2}{3}$  and 5 $\frac{2}{3}$  days, whereas in the theocin series 6 of the 7 animals died inside of 4 $\frac{2}{3}$  days, and in the caffein and potassium acetate



series 5 died inside of 5 days, and of these early deaths in each series 3 died on the 4th day. The spartein series parallels fairly closely the water series. Therefore, we may consider that water in such cases is detrimental to a certain extent in general, and may be very harmful in some cases. This substantiates more or less that diuretics (water being considered a diuretic) are detrimental in acute nephritis, but water is not as harmful as the diuretic drugs.

This brings up the question, are these drugs in themselves toxic? The next series was used to study the toxicity of the drugs and also of water alone in such large amounts when given intravenously to normal animals.

TABLE 3.—EFFECT OF STERILE WATER INJECTIONS IN URANIUM NEPHRITIS

Rabbit No.	Weight	Result	Treatment	C.C. Fluid	No Doses	Animals Dying on Successive Days after First Uranium Injection					
						2 1/3 Days	3 1/3 Days	4 Days	4 2/3 Days	5 2/3 Days	Average No. Days
639	2360	died	Ster. H <sub>2</sub> O	9.5	6	....	....	....	1	....	.....
640	2060	died	Ster. H <sub>2</sub> O	8.	6	....	....	....	1	....	5 1/6
641	2300	died	Ster. H <sub>2</sub> O	9.	8	....	....	....	....	1	.....
642	2260	died	Ster. H <sub>2</sub> O	9.	8	....	....	....	....	1	.....
635	2050	died	Controls	.....	.....	....	....	....	1	....	.....
636	1850	died	Controls	.....	.....	....	....	....	....	1	.....
637	2150	lived	Controls	.....	.....	....	....	....	....	....	.....
638	2600	lived	Controls	.....	.....	....	....	....	....	....	.....

FOURTH SERIES OF EXPERIMENTS

The fourth series consists of 12 normal rabbits, to 4 of which sterile water in doses of 4 c.c. per kilo of body weight was given intravenously, and to every 2 of the remaining 8 rabbits was given intravenously theocin, caffein, potassium acetate and spartein sulphate, respectively, using the same amounts of drug and water per kilo of body weight as was used in the first series of 33 animals which had an acute nephritis. All of these 12 rabbits survived 4 days of twice daily intravenous injections; that is, eight injections altogether, and all appeared normal and none the worse for the treatment, except that their ear veins were so thrombosed and injured that intravenous injections had to be discontinued. These rabbits received more intravenous injections than did the nephritis series and survived them in good condition, whereas the rabbits in the first series showed signs of failure usually a day before death, and

they died with seven or less injections. Consequently this series would seem to prove that the water alone and the diuretic drugs alone were not toxic when given intravenously in large doses to normal animals, over a reasonable length of time.

Histological study of the kidneys of all of the rabbits that died showed a severe grade of nephritis with but little variation in appearance in the different kidneys.

#### CONCLUSIONS

We feel that the following conclusions may be safely considered from this work and that they suggest a similar effect in man.

1. The diuretic drugs, theocin, caffein and potassium acetate definitely shorten the life of a rabbit having a severe acute experimental nephritis produced by uranium nitrate.

2. Theocin and potassium acetate gave quite parallel results and were slightly more harmful than caffein.

3. Spartein sulphate although not nearly so detrimental as the other drugs, however, did, in some cases, shorten the animal's life.

4. Water in large amounts is detrimental in some cases, possibly depending on the severity of the nephritis.

5. The diuretics alone in large doses and water alone in large doses when given intravenously to normal animals for a reasonable length of time do not shorten their life and probably are not toxic in themselves.

6. Diuretics are probably contra-indicated in severe acute nephritis in man, since in animals in such cases they shorten life.

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## A CONTRIBUTION TO THE BACTERIOLOGY OF THE DUODENUM \*

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Escherich,<sup>1</sup> in his study of intestinal bacteria in children, found that the small intestine during the intervals when only intestinal secretions are present is almost free from bacteria. Tissier<sup>2</sup> examined the duodenal fluid obtained at autopsy from children dying without digestive derangement. He found in his cultures numerous colonies of *B. coli* and the enterococcus of Thiercelin, together with other organisms in small numbers. Experimental work on animals and observations on men undergoing surgical operations by Cushing and Livingood<sup>3</sup> and by Kohlbrugge,<sup>4</sup> has shown that the small intestine becomes practically germ-free in the absence of food material, and speaks of autosterilization of the small intestine. The factors on which this autosterilization depend are not definitely known.

In adults the bacteria of the duodenum have been studied by Gessner<sup>5</sup> in eighteen cases in which autopsy was performed soon after death. Cases of digestive disturbance were excluded from the series. Gessner found an organism probably identical with *B. lactis aerogenes* in large numbers in eight cases, *B. coli* very frequently, two other types of bacilli, two kinds of staphylococci and one variety of streptococcus. Quantitative data were not obtained.

Hess<sup>6</sup> has studied the bacteria in the duodenal fluid of infants, employing a duodenal catheter to obtain the fluid. He found the staphylococcus to be the most common organism in the duodenal fluid of young infants. In examining fifteen cases he found only once the colon bacillus, or, indeed, any gas-producing organism, a result distinctly different from that obtained by previous study of the duodenal fluid obtained at autopsy. Hess suggests the use of the duodenal tube in studying the duodenal flora of adults, especially for the purpose of detecting the presence of typhoid bacilli.

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\* Submitted for publication May 3, 1913.

1. Escherich: *Die Darmbakterien des Säuglings*, 1886.

2. Tissier: *Ann. de l'Inst. Pasteur*, 1905, xix, 109.

3. Cushing and Livingood: *Contributions to the Science of Medicine* dedicated to W. H. Welch, 1900, p. 543.

4. Kohlbrugge: *Centralbl. f. Bakteriol.*, 1901, xxix, 571; xxx, 10 and 70.

5. Gessner: *Arch. f. Hyg.*, 1889, ix, 128.

6. Hess: *Jour. Infect. Dis.*, 1912, ii, 71.

Our own observations were undertaken in order to perfect a technic by which duodenal fluid suitable for bacteriological study could be obtained from adults and to gain some knowledge concerning the microbes present in the duodenum in health and in gastro-intestinal disturbances.

#### TECHNIC OF OBTAINING DUODENAL CONTENTS

The duodenal tube consists of a small soft rubber tube, external diameter 3.5 mm., bearing at the lower end a perforated gold tip. This tube is sterilized by boiling in water for at least ten minutes. Before introducing the tube a gelatin capsule, which had been soaked for several days in 95 per cent. alcohol, is picked up with sterile forceps and tightly fitted over the gold tip of the tube. The tube with the gelatin capsule in place is dipped in shellac and dried several times until it is well covered. The coating of shellac prevents the gelatin capsule from being dissolved in the stomach. Several other methods were tried before the above was adopted; soaking the capsule in formaldehyd solution rendered it so hard that it would not dissolve in the duodenal juice; covering the tube with shellac without the use of the gelatin capsule filled up the perforations of the gold tip so effectually that the duodenal juice could not penetrate them; the use of thin rubber tissue over the tip of the tube and bursting it by inflation after the tube was in place was found to be impracticable.

After the tube is sterilized and the gelatin capsule in place and well covered with shellac, it is placed in the patient's mouth with instructions for him to swallow it. With the patient lying on his right side the tube is usually carried by the peristaltic waves of the stomach into the duodenum in about twenty minutes. Its position can be demonstrated either by the fluoroscope or by examination of the aspirated contents. The tube is usually given at half past 10 at night and the duodenal contents aspirated with a sterile glass syringe at about nine in the morning; then, with the tube in place, an Ewald test-breakfast is given and the duodenal contents aspirated one hour later. Patients sometimes object to taking the test-breakfast with the duodenal tube *in situ*. Occasionally, the tube is passed at 7 a. m., and the duodenal contents aspirated at 9 a. m. This method is more acceptable to the patient and seems to give as accurate results as the introduction of the tube at night.

The laboratory study of the duodenal fluids<sup>7</sup> consists of macroscopic and microscopic examination of the fluid, a direct microscopic count of the bacterial cells by the Winterberg method, quantitative inoculation of cultures on litmus-lactose-agar plates, ascitic-fluid-agar plates, shake-tube cultures according to the method of Veillon<sup>8</sup> in ascitic-glucose agar,

7. For a more detailed consideration of the methods see MacNeal, Latzer and Kerr, *Jour. Infect. Dis.*, 1909, vi, 123 and 571.

8. Veillon and Zuber: *Arch. de méd. exper.*, 1898, series 1, x, 517.



and cultures in fermentation tubes of glucose broth and lactose broth. In addition, a portion of the fluid is heated to 80 C. for ten minutes and inoculated in measured quantities into Veillon tubes of ascitic-fluid-glucose-agar and fermentation tubes of glucose and lactose broth for the detection of spores.

#### DETAILED RESULTS OF EXAMINATION

The results obtained with the first nine samples of fluid are omitted from this paper because of various faults in technic, relating more particularly to the removal of the material from the patient. There remain twenty-six fluids derived from twenty-four different patients, each of two patients having been examined twice.

*Fluid 10* was removed from the patient R. L., 22 years old, who had suffered from alcoholic neuritis for the past year. He complained of severe pain in the abdomen and lower chest. His general nutrition was good and the analysis of his gastric contents after an Ewald test-breakfast was practically normal—total acidity 58 and free hydrochloric acid 28, expressed as c.c. of decinormal acid per 100 c.c. of gastric juice. Analysis of his urine and examination of his feces showed no signs of gastro-enteric disturbance.

The duodenal tube was introduced to a distance of 85 cm. from the incisor teeth at 10:30 p. m. November 7. The next morning an Ewald test-breakfast was given at 8 o'clock and at 9 a. m. 25 c.c. of duodenal contents were aspirated by means of a sterile glass syringe. Part of this, 15 c.c., was used for chemical study, which showed the presence of lipase, trypsin and bile. The remainder was subjected to bacteriological examination.

The latter specimen consisted of approximately 10 c.c. of a greenish-brown fluid with an abundant greenish-yellow sediment, amounting to about 2.5 c.c. in the bottom of the tube. It was odorless. Microscopically the sediment was found to consist of starch grains and a moderate number of yeast cells. The direct microscopic count showed 132,500 bacterial cells per milligram of fluid, most of them staining very feebly with the methylene blue. The plates of litmus lactose agar inoculated with 0.1 c.c. and 0.002 c.c. of fluid remained sterile after four days at 37 C. The plate of ascitic-fluid agar inoculated with 0.1 c.c. showed one colony, a Gram-positive, gelatin-liquefying coccus, Strain 1. The Veillon tubes and fermentation tubes showed no growth.

*Fluid 11* was obtained from the patient C. L., aged 32, who had suffered from gastric ulcer for the previous six months. Soreness in the epigastrium and pain immediately after meals were the most prominent symptoms. He was a fireman by occupation and was well developed physically. His stomach contents showed total acidity, 75, and free hydrochloric acid 50. Examinations of the urine and feces were negative.

The duodenal tube was introduced at 10:30 p. m. Nov. 6, 1912, to the distance of 85 cm. from the incisor teeth. At 8 a. m., November 7, an Ewald test-breakfast was given and, one hour later, 15 c.c. of duodenal contents were aspirated by means of a sterile glass syringe. Part of this, about 8 c.c., was examined chemically. Its total acidity to phenolphthalein was 25; lipase and trypsin were present. The remainder was used for the bacteriological examination.

There were 7 c.c. of a greenish-yellow fluid containing a finely divided yellow sediment occupying the lower 2 c.c. The supernatant fluid was opalescent and odorless. Microscopic examination showed many starch grains, a moderate number of yeasts and crystals of cholesterol. The direct microscopic count showed 232,000 bacteria per milligram, most of them staining well with the

methylene blue, and 12,000 yeast cells per milligram. On the plates of litmus lactose agar 1,630 bacteria per c.c. developed into colonies, and on the ascitic-fluid agar 930 per c.c. Two of these colonies, one a micrococcus and one a bacillus, were transplanted and designated as Strains 2 and 3, respectively. The fermentation tubes gave positive growth, but there was no production of gas after five days. The Veillon tubes developed 1,200 colonies per c.c. Cultures inoculated with the heated fluid (spore material) gave no growth.

*Fluid 12* was obtained from the patient G. H., female, 27 years old, who had suffered from duodenal ulcer with the usual symptoms for the previous year. Gastric analysis showed the total acidity to be 37 and free hydrochloric acid 10. The examinations of urine and feces were negative. The duodenal tube was introduced at 10:30 p. m., November 8, to the distance of 85 cm. At 9:30 a. m., November 9, without the ingestion of any food in the meantime, 13 c.c. of duodenal contents were aspirated. The chemical examination showed a total acidity of 8, and the presence of amylase, lipase and trypsin. A portion of the fluid was used for bacteriological examination.

The sample of fluid amounted to 3.5 c.c. It was orange yellow and clear except for a small amount of white flaky material. There was no perceptible odor. Microscopic examination of the sediment showed mucous threads, a few small flat crystals, apparently cholesterol, and a moderate number of bacteria. By the direct microscopic method 2,500 bacterial cells per milligram were counted. On the plates of litmus lactose agar 160 bacteria per c.c., and on the plates of ascitic-fluid agar 1,144 bacteria per c.c. developed into colonies. Three colonies were transplanted from the latter set of plates and the bacterial strains designated as 4, 5 and 6. The fermentation-tube cultures remained free from gas after eight days. The Veillon tubes of ascitic-fluid glucose agar brought to development about 1,200 bacteria per c.c. of fluid inoculated. The tubes inoculated with spore material remained sterile.

*Fluid 13* was obtained from the patient S. W., 52 years old, suffering from a mild attack of cholecystitis, probably with gall-stones. The pains were general over the right hypochondrium. The patient was well nourished and in good physical condition. Gastric analysis showed total acidity 60, and free hydrochloric acid 30. Urine analysis was negative. The feces were very acid and contained much undigested material. The duodenal tube was introduced at 10:30 p. m., November 8, to the distance of 95 cm. from the incisor teeth. An Ewald test-breakfast was given at 9 a. m., November 9, while the tube remained in position, and at 10 a. m. 12 c.c. of duodenal contents were aspirated. The chemical analysis showed total acidity 10 (phenolphthalein), and the presence of lipase, trypsin and bile. The major portion of the fluid was used for the bacteriological examination.

There were approximately 10 c.c. of lemon yellow viscous fluid. Consistency was about that of the white of an egg. It was almost perfectly clear. Microscopic examination of the sediment showed a few yeasts and bacterial cells. By the direct microscopic count 600 bacteria per milligram were found. On the plates of litmus lactose agar and ascitic-fluid agar only 4 bacteria per c.c. developed into colonies. Two colonies were transplanted from the latter set of plates and the cultures designated as Strains 7 and 8. The fermentation-tube cultures showed no production of gas and the Veillon tubes remained sterile. The cultures inoculated with spore material also remained sterile.

*Fluid 14* was obtained from the patient M. G. at 9:30 a. m., Nov. 11, 1912. The patient was a woman 36 year old suffering from a gastric ulcer for the previous two years. Radiographs showed an hour-glass contraction of the stomach. There was an area of exquisite tenderness over the epigastrium. The patient was under nourished and moderately anemic. She had lost several pounds in weight. No free hydrochloric acid was present in the gastric juice and the total acidity of it was 8. Examinations of urine and feces were negative. The duodenal tube was introduced at 10:30 p. m., November 10, to the distance

of 98 cm. No food was taken after 6 p. m., November 10. Between 9:15 a. m. and 9:50 a. m., November 11, 19 c.c. of duodenal contents were aspirated. The chemical analysis showed a total acidity of 8 (phenolphthalein) and the presence of amylase, lipase and trypsin. A part of the fluid was used for bacteriological examination.

The latter portion measured about 8 c.c. and consisted of an odorless, slightly smoky, yellow fluid containing a small amount of flocculent precipitate, distributed throughout the fluid. There was a little foam on the surface. Microscopic examination of the sediment showed large numbers of bacteria and, by direct microscopic count, 44,300 per milligram of fluid were found. On the plates of litmus lactose agar 9,200,000, and on the ascitic-fluid agar 3,900,000 bacteria per c.c. of fluid developed into colonies. Strains 9, 10, 11, 12 and 13 were taken from the former, and Strains 14, 15 and 16 from the latter set of plates. Glucose broth and lactose broth in fermentation tubes, inoculated with 0.1 c.c., 0.002 c.c. and 0.0002 c.c. was fermented with the production of 40 to 75 per cent. of gas, and in one tube there was 100 per cent. gas in the closed arm. The Veillon tubes developed colonies too numerous to count, most of them near the free surface. The tubes inoculated with spore material remained sterile.

*Fluid 15* was obtained from patient T. B., male, aged 34, suffering for the previous ten years from duodenal ulcer. During this period he had free intermissions between attacks of intense pain and hemorrhage into the bowel. Gastric analysis showed total acidity 89, and free hydrochloric acid 70. There was no food retention in the stomach over night. Examinations of the urine and feces were negative. The duodenal tube was introduced at 10 p. m., November 11, to the distance of 83 cm. The last food was taken at 6 p. m., November 11. November 12 at 9 a. m. 9 c.c., at 9:10 a. m. 7 c.c., and at 10 a. m. 3 c.c. of the duodenal contents were aspirated. The chemical analysis of the first portion aspirated showed total acidity 5 (phenolphthalein) and the presence of amylase, lipase and trypsin. The second portion, aspirated at 9:10 a. m., showed a total acidity of 15 and again amylase, lipase and trypsin. The fluid withdrawn at 10 a. m. was used for the bacteriological examination.

The sample consisted of about 3 c.c. of almost clear, golden yellow fluid with a small amount of mucoid sediment. A slight odor suggestive of fresh fish was noted. Microscopic examination of the sediment showed very little formed material, a few irregular flat crystals, probably cholesterin, and some very finely divided vegetable material. Direct microscopic count showed 6,800 bacterial cells per milligram. On the plates of litmus lactose agar 10 bacteria per c.c., and on ascitic-fluid agar 70 bacteria per c.c. developed into colonies. Two colonies were transplanted from the latter series of plates and designated as Strains 17 and 18. All the fermentation-tube cultures remained free from gas. Of the Veillon tubes, one developed seven colonies and another a single colony. These tubes were each inoculated with 0.1 c.c. of the fluid. Tubes inoculated with smaller quantities remained sterile. Strains 19 and 20 were taken from the colonies in these tubes. The media inoculated with spore material remained sterile.

*Fluid 16* was obtained from patient B. D., female, aged 24, suffering for the past six months from obstinate constipation with severe colonic pain and persistent headache. The radiograph showed the transverse colon to be prolapsed as far down as the pelvis. The patient had a pasty complexion but was well nourished. The gastric contents after an Ewald test-breakfast showed total acidity 50, and free hydrochloric acid 15. Examinations of the urine and feces were negative. The duodenal tube was introduced at midnight November 12 to the distance of 85 cm. At 9:30 a. m., without ingestion of food during the interval, 6 c.c. of duodenal contents were aspirated. The chemical examination showed total acidity 15 (phenolphthalein), and the presence of amylase, lipase



and trypsin. A small amount of the fluid was used for bacteriological examination.

The specimen consisted of approximately 1 c.c. of clear, golden yellow fluid with a small amount of sediment. Microscopic examination of the sediment showed bacilli and micrococci. By the direct microscopic counting method 148,250 bacteria per milligram of fluid were found. Plates inoculated with 0.1 c.c. and 0.002 c.c. of the fluid remained sterile, and the fermentation tubes remained free from gas. One of the Veillon tubes inoculated with 0.1 c.c. of fluid showed a surface growth, which was transplanted and designated as Strain 21. The tubes inoculated with spore material remained free from growth.

*Fluid 17* was obtained from the patient F. S., male aged 43, suffering from intestinal and pulmonary tuberculosis. Four years previously he had had about 8 inches of the ileum removed for relief of a tuberculous stricture. Tuberculosis recurred in his intestine and became manifest in his lungs about three years later. The patient was anemic and had lost several pounds in weight. Gastric analysis showed total acidity 35, and free hydrochloric acid 20. Examinations of the feces and urine were negative. The duodenal tube was introduced at 10:30 p. m., November 13, to the distance of 83 cm. At 9:10 a. m., without ingestion of food in the interval, 12 c.c. of duodenal contents were aspirated. Chemical examination showed total acidity 13 (phenolphthalein), and the presence of amylase, lipase and trypsin. A small portion of the material was reserved for bacteriological study.

The sample consisted of approximately 3 c.c. of an almost perfectly clear, golden yellow, odorless fluid. Microscopic examination of the sediment showed micrococci and some unidentified debris. By the microscopic counting method 67,000 bacterial cells per milligram of fluid were found. Special microscopic examination for acid-proof bacilli was negative. Of the two sets of plates inoculated with 0.1 c.c. and 0.0002 c.c. of fluid, only the one plate of ascitic-fluid agar inoculated with 0.1 c.c. gave any growth. This showed three colonies, equivalent to 30 bacteria per c.c. of fluid. Two of these colonies were transplanted and the cultures designated as Strains 22 and 23. All the fermentation tubes remained free from gas. The Veillon tubes inoculated with 0.1 c.c. and 0.0002 c.c. each developed a single colony. These were transplanted and designated as Strains 24 and 25. The tubes inoculated with spore material remained sterile.

*Fluid 18* was obtained from the patient J. F., male, aged 24, convalescing from an attack of influenzal pneumonia of two weeks' duration. The patient was fairly well nourished. Gastric analysis was not made. Examinations of the urine and feces were negative.

The duodenal tube was introduced at midnight November 13 to the distance of 63 cm. At 9:30 a. m., without the ingestion of any food in the meantime, 15 c.c. of duodenal contents were aspirated. The chemical examination showed total acidity 20 (phenolphthalein), and the presence of amylase, lipase and trypsin. A part of the fluid was used for bacteriological examination.

The sample for bacteriological study was approximately 3 c.c. of greenish-yellow, cloudy fluid, containing some flakes of mucus. Microscopic examination of the sediment showed only unidentified debris. By the direct counting method 125,000 bacterial cells per milligram of fluid were found. Special microscopic examination for acid-proof bacilli gave a negative result. On the plates of ascitic-fluid agar 3,400,000 bacteria per c.c. developed into colonies, and on the litmus lactose agar 2,300 per c.c. The fermentation tube of glucose broth inoculated with 0.1 c.c. produced a small bubble of gas and the lactose broth inoculated with the same amount produced 30 per cent. gas in the closed arm. Those inoculated with 0.0002 c.c. and with 0.1 c.c. of spore material produced no gas. The Veillon tubes, even those inoculated with 0.0002 c.c. of the fluid, developed colonies too numerous to count. Subculture Strains 26, 27, 28, 29 and 30 were



taken from the plates of ascitic-fluid agar and 31 and 32 from the plates of litmus lactose agar.

*Fluid 19* was obtained from the patient C. F., female, 25 years old, suffering from neuritis, with indefinite pains over the abdomen. The patient had well developed hysteria. Gastric analysis showed total acidity 54, and free hydrochloric acid 30. Examinations of the urine and feces were negative.

The duodenal tube was introduced at 10:30 p. m., November 14, to the distance of 79 cm. The next morning at 9:30 a. m., without ingestion of food in the meantime, 10 c.c. of duodenal contents were aspirated. The chemical examination showed a total acidity of 10 (phenolphthalein), and the presence of amylase, lipase and trypsin.

The specimen for bacteriological study consisted of approximately 5 c.c. of odorless, cloudy, golden yellow fluid. Microscopic examination showed bacteria and yeasts amounting to 366,000 bacterial cells, and 1,320 yeast cells per milligram of fluid. Plates inoculated with 0.1 c.c. and 0.0002 c.c. remained free from colonies, except the plate of ascitic-fluid agar inoculated with 0.0002 c.c., on which two surface colonies developed. Although these probably represented contaminations they were transplanted and the strains designated as 33 and 34. The fermentation tubes and Veillon tubes inoculated with 0.1 c.c. and 0.0002 c.c. remained sterile.

*Fluid 20* was obtained from the patient W. D., male, aged 18, suffering from a typhoid relapse. The duodenal tube was introduced at 10:30 p. m., November 14. The next morning the patient drank milk while the tube remained in position, and at 11 a. m. about 2 c.c. of duodenal contents were aspirated. It was used for the bacteriological examination.

There were approximately 2 c.c. of cloudy, greenish-yellow fluid, with a slight odor suggesting cheese. The direct microscopic count showed 775,000 bacteria per milligram of fluid. Plate cultures on ascitic-fluid agar brought to development 1,600,000, and on litmus lactose agar 1,100,000 bacteria per c.c. of fluid. From the former set of plates six colonies were transplanted and preserved as Strains 35, 36, 37, 38, 39 and 40. Seven alkaline colonies were transplanted from the plates of litmus-lactose agar and these strains were designated as 41, 42, 43, D 20(1), D 20(2), D 20(3) and D 20(4). The fermentation tube of glucose broth inoculated with 0.05 c.c. of the fluid showed 10 per cent. gas in the closed arm after four days and that of lactose broth showed 75 per cent. gas. The fermentation tubes inoculated with 0.0002 c.c. of the fluid and with 0.1 c.c. of spore material remained free from gas. The Veillon tubes inoculated with 0.05 c.c. and 0.0002 c.c. of the fluid developed colonies too numerous to count. The one inoculated with spore material (0.06 c.c.), showed no growth.

*Fluid 21* was obtained from the patient J. K., female, aged 64, suffering from atrophic gastritis.<sup>9</sup> She complained of anorexia, vomiting and general weakness. Gastric analysis showed total acidity 10, and free hydrochloric acid 0. There were a few pus cells in the gastric lavage. Examinations of feces and urine were negative.

The duodenal tube was introduced at 10:30 p. m., November 15, to the distance of 99 cm. At 12:30 noon, November 16, without ingestion of any food in the meantime, 5 c.c. of duodenal contents were aspirated. Chemical analysis showed total acidity 10, and the presence of amylase, lipase and trypsin.

The portion for bacteriological study was approximately 5 c.c. of cloudy, golden-yellow fluid, with a flaky sediment. The microscopic findings and the direct bacterial count were not recorded. Plate cultures on ascitic-fluid agar brought to development 1,000,000 bacteria and on litmus lactose agar, 1,600,000 bacteria per c.c. of fluid. The usual fermentation-tube cultures of lactose broth were not inoculated with this fluid. In the fermentation tube of glucose broth

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9. At the time of this examination the condition was diagnosed as gastric carcinoma. The subsequent history of the case was such that the diagnosis has been recognized as in all probability erroneous.

inoculated with 0.05 c.c. of the fluid, 30 per cent. of gas was produced. Similar tubes inoculated with 0.0002 c.c. of the unheated fluid and with 0.02 c.c. of spore material showed no gas. The Veillon tubes inoculated with 0.05 c.c. and 0.0002 c.c. of the fluid developed colonies too numerous to count; those inoculated with spore material remained sterile. Five subcultures taken from colonies on the plates of litmus lactose agar were designated as Strains 44, 45, 46, 47 and 48.

*Fluid 22* was obtained from the patient M. G., at 12:15 noon, Nov. 16, 1912, the same patient from whom Fluid 14 had been obtained November 11. See page 181.

The duodenal tube was introduced at 10:30 p. m., November 15, to the distance of 99 cm. At 12:15 noon, November 16, about 3 c.c. of duodenal contents were aspirated. The patient had fasted while the tube remained in position. The specimen was used for bacteriological examination.

There were approximately 3 c.c. of clear, golden-yellow fluid. The results of the microscopic examination of the sediment and of the direct bacterial count were not recorded. On the plates of ascitic-fluid agar 85,000,000 bacteria per c.c. of fluid developed into colonies, and on the litmus lactose agar 31,000,000. Two colonies from the latter set of plates were transplanted and the strains preserved as 49 and 50, and three strains taken from colonies on the plates of ascitic-fluid agar were designated as 51, 52 and 53. Fermentation tubes of lactose broth were not inoculated. Fermentation tubes of dextrose broth, inoculated with 0.1 c.c. and 0.0002 c.c. of the fluid, produced 60 per cent. and 40 per cent. of gas, respectively. The Veillon tubes inoculated with 0.1 c.c. and 0.0002 c.c. of fluid developed colonies too numerous to count. The tubes inoculated with spore material showed no growth.

*Fluid 23* was obtained from the patient R. E., female, aged 40, suffering from general enteroptosis and cystitis. Analysis of the gastric juice showed total acidity 27, and free hydrochloric acid 13. The urine contained much pus. Examination of the feces was negative.

The duodenal tube was introduced at 10:30 p. m., November 17, to the distance of 77 cm. The patient fasted until 9:30 a. m., November 18, when about 8 c.c. of duodenal contents were aspirated. The chemical examination showed total acidity 5 (phenolphthalein), and the presence of lipase and trypsin. A portion of the sample was used for bacteriological examination.

The specimen consisted of approximately 3 c.c. of cloudy, yellow fluid. By the direct microscopic counting method 124,000 bacteria per milligram of fluid were found. On the plates of litmus lactose agar 150 bacteria per c.c. developed into colonies and on the ascitic-fluid agar 550 per c.c. Three colonies were transplanted from the ascitic-agar plates and the strains designated as 54, 55 and 56. Four strains were taken from the litmus lactose agar. These were designated as 57, 58, 59 and 60. Fermentation tubes of glucose broth and lactose broth inoculated with 0.1 c.c. and 0.0002 c.c. of the fluid produced no gas. Veillon tubes of ascitic-fluid agar brought to development 200 bacteria per c.c. The tubes inoculated with spore material remained sterile. Two colonies were transplanted from the Veillon tubes and the cultures designated as Strains 61 and 62.

*Fluid 24* was obtained from the patient E. H., female, aged 27, suffering from myasthenia gastrica and splanchnoptosis. This condition had persisted for several years. The patient was anemic but fairly well nourished. There was no gastric retention of food over night. Gastric analysis showed total acidity 56, and free hydrochloric acid 24. Examinations of the urine and feces were negative.

The duodenal tube was introduced at 10:30 p. m., November 17, to the distance of 85 cm. The patient fasted until 9 a. m., November 18, when 10 c.c. of duodenal contents were aspirated. Chemical analysis showed total acidity 5, and the presence of amylase, lipase and trypsin. A small portion of the sample was used for bacteriological examination.

This portion consisted of approximately 2 c.c. of perfectly clear, golden-yellow fluid. By the direct microscopic method 496,000 bacterial cells per milligram of the fluid were counted. Plate cultures on ascitic-fluid agar brought to development 130 bacteria per c.c. and cultures on litmus lactose agar brought to development 80 per c.c. Three of the colonies on the ascitic-fluid agar were transplanted and preserved as Strains 64, 65 and 66. One colony was transplanted from the litmus lactose agar and designated as Strain 67. Fermentation tubes inoculated with 0.05 c.c. and 0.0002 c.c. of the fluid remained free from gas. Veillon tubes brought to development 50 bacteria per c.c. of the fluid. The tubes inoculated with spore material remained sterile. One colony was transplanted from one of the Veillon tubes and designated as Strain 63.

*Fluid 25* was obtained from the patient W. D., male, aged 42, suffering from hypertrophic cirrhosis of the liver. No gastric analysis was made. The urine contained much bile and the feces a diminished amount of bile pigment.

The duodenal tube was introduced at 10:30 p. m., November 18, to the distance of 88 cm. No food was taken until after 9:15 a. m., November 19, when 33 c.c. of duodenal contents were aspirated. Chemical analysis showed total acidity 3, and the presence of amylase, lipase and trypsin. The remainder of the fluid was used for bacteriological examination.

This portion consisted of approximately 23 c.c. of a clear golden-yellow fluid. Direct microscopic count showed 78,000 bacteria per milligram of the fluid. Plate cultures on ascitic-fluid agar brought to development 13,600,000 and on litmus lactose agar 2,900,000 bacteria per c.c. Four colonies were transplanted from the ascitic-fluid agar and designated as Strains 68, 69, 70 and 71, and two colonies transplanted from the litmus lactose agar were designated as Strains 72 and 73. Fermentation-tube cultures inoculated with 0.02 c.c. and 0.0002 c.c. of the fluid remained free from gas. The Veillon tubes developed colonies too abundant to count. The tubes inoculated with spore material remained sterile.

*Fluid 26* was obtained from the patient J. A., male, aged 20, suffering from persistent epigastric pain. The liver and spleen were markedly enlarged and the cause of this enlargement was not ascertained. The condition was diagnosed as gastric ulcer. Gastric analysis showed total acidity 20, and free hydrochloric acid 5. Examinations of the feces and urine were negative.

The duodenal tube was introduced at 10:30 p. m., November 18, to the distance of 91 cm. No food was taken until after 9 a. m. on November 19, when 1 c.c. of duodenal contents was aspirated. This was used for bacteriological examination.

The sample consisted of approximately 1 c.c. of cloudy, golden-yellow fluid. Eight hundred sixty-four thousand bacterial cells per milligram were counted microscopically. Plate cultures on ascitic-fluid agar brought to development 850 and on litmus lactose agar 150 bacteria per c.c. of fluid. Two colonies were transplanted from the ascitic-fluid agar and designated as Strains 74 and 75, and two from the litmus lactose agar were designated as Strains 76 and 77. Fermentation tubes of glucose and lactose broth inoculated with the fluid remained free from gas. Veillon tubes brought to development 350 bacteria per c.c. of fluid. Tubes inoculated with spore material remained sterile. Three colonies were transplanted from the Veillon tubes and the subcultures designated as Strains 78, 79 and 80.

*Fluid 27* was obtained from the patient B. B., male, aged 34, suffering from the recurrence of a chronic gastric ulcer. About one year before a gastro-enterostomy had been performed because of stenosis of the pylorus due to the ulcer. He suffered intense epigastric pain which was somewhat relieved by vomiting. He had lost several pounds in weight and was anemic. Gastric analysis showed total acidity of 105 and free hydrochloric acid 90. Examinations of the urine and feces were negative.



The duodenal tube was introduced at 10:30 p. m., November 20, to the distance of 80 cm. At 10 a. m., November 21, before any food was taken, 60 c.c. of duodenal contents were aspirated. Chemical analysis showed total acidity 0, and the presence of amylase, lipase and trypsin. About a third of the fluid was used for bacteriological examination.

This portion consisted of approximately 20 c.c. of a very clear, golden-yellow fluid. Microscopic examination showed practically nothing but bacterial cells, which numbered 10,000 per milligram of the fluid. Plate cultures on ascitic-fluid agar brought to development 3,000, and on litmus-lactose agar 340 microbes per c.c. of fluid. Four colonies were transplanted from the ascitic-fluid agar and preserved as Strains 81, 82, 83 and 84. Fermentation-tube cultures of glucose broth and lactose broth inoculated with 0.1 c.c. of the fluid produced 60 per cent. and 25 per cent. gas, respectively. Similar tubes inoculated with 0.0002 c.c. of the fluid remained free from gas. The Veillon tubes developed enormous numbers of colonies and these were not counted. Tubes inoculated with spore material showed no growth.

*Fluid 28* was obtained from the patient H. G., male, aged 29, suffering from atrophic gastritis. He complained of heaviness in his epigastrium and persistent bad taste. Gastric analysis showed total acidity 12, and free hydrochloric acid 0. Examinations of feces and urine were negative.

The duodenal tube was introduced at 10:30 p. m., November 20, to the distance of 85 cm. No food was taken before 9:50 a. m., November 21, at which time 8 c.c. of duodenal contents were aspirated. The chemical examination showed total acidity 5, and the presence of amylase, lipase and trypsin. Half of the fluid was used for the bacteriological examination.

There was approximately 4 c.c. of a fluorescent golden-yellow fluid. The microscopic examination was negative except for bacteria, of which 30,000 per milligram of the fluid were counted. Plate cultures on ascitic-fluid agar brought to development 120, and on litmus lactose agar 50 bacteria per c.c. Three colonies were transplanted from the ascitic-fluid agar and the strains designated as 85, 86 and 87. One subculture taken from the litmus lactose agar was designated as 88. The fermentation tube of lactose broth inoculated with 0.1 c.c. of the fluid produced 75 per cent. gas and that of glucose broth inoculated with a similar amount produced 5 per cent. Similar tubes inoculated with 0.0002 c.c. of the fluid remained free from gas. The Veillon tubes brought to development 150 bacteria per c.c. of fluid; one of these colonies was transplanted and preserved as Strain 89. Tubes inoculated with spore material remained sterile.

*Fluid 29* was obtained from patient L. L., male, aged 23, suffering from a tuberculous pyelitis. No gastric analysis was made. The urine contained blood and pus. Examination of the feces was negative.

The duodenal tube was introduced at 10:30 p. m., November 24, to the distance of 90 cm. No food was taken while the tube was in position. At 9.15 a. m., November 25, 20 c.c. of duodenal contents were aspirated. The chemical examination showed total acidity 10, and the presence of amylase, lipase and trypsin. A small portion was used for bacteriological study.

This part consisted of approximately 3 c.c. of clear, smoky, golden-yellow fluid. One hundred fourteen thousand bacterial cells per milligram were counted by the direct microscopic method. Special microscopic examination for acid-proof bacilli gave a negative result. Plate cultures on ascitic-fluid agar brought to development 1,600,000 bacteria per c.c., and on litmus lactose agar only 80 bacteria per c.c. Four colonies were transplanted from ascitic-fluid agar and preserved as Strains 90, 91, 92 and 94, the number 93 seeming to have been inadvertently omitted. Fermentation-tube cultures remained free from gas. The Veillon tubes brought to development 1,000 bacteria per c.c. of the fluid. Two of these colonies were transplanted and designated as Strains 95 and 96. Tubes inoculated with spore material showed no growth.



*Fluid 30* was obtained from the patient A. N., male, aged 48, suffering for several years from the symptoms of asthenic gastritis and from constipation, with much pain in the lower abdomen. Gastric analysis showed total acidity 14, and free hydrochloric acid 4. The urine contained a trace of indican. Examination of the feces was negative.

The duodenal tube was introduced at 10:30 p. m., November 25, to the length of 102 cm. No food was taken while the tube was in position. At 9:15 a. m., November 26, 10 c.c. of duodenal contents were aspirated. The chemical examination showed total acidity 5, and the presence of amylase, lipase and trypsin. A small portion of the sample was used for the bacteriological examination.

This portion was approximately 1 c.c. of clear, golden-yellow fluid with a very small amount of suspended material. Microscopic examination showed bacterial cells which numbered 33,400 per milligram according to the direct microscopic count. Plate cultures on ascitic-fluid agar brought to development 2,600 and on litmus lactose agar 2,400 bacteria per c.c. Two colonies were transplanted from the litmus lactose agar and the cultures designated as Strains 97 and 98, 99 and 100. Fermentation tubes of glucose and lactose broth inoculated with 0.05 c.c. of the fluid produced a small bubble of gas in each. Similar tubes inoculated with 0.0002 c.c. remained free from gas. The Veillon tubes brought to development a large number of bacteria, approximately 10,000 per c.c. of the fluid. Two of these colonies were transplanted and the subcultures designated as Strains 101 and 102.

*Fluid 31* was obtained from patient K. H., female, aged 48, suffering from the symptoms of gastric neurosis and asthenic gastritis, with loss of weight and a feeling of heaviness in the epigastrium after meals. Gastric analysis showed total acidity 32, and free hydrochloric acid 13. Examinations of the feces and urine were negative.

The duodenal tube was introduced at 10:30 p. m., November 28, to the length of 80 cm. No food was taken while the tube remained in position. At 9:15 a. m., November 29, 2 c.c. of duodenal contents were aspirated, and this was used for the bacteriological examination.

The sample consisted of approximately 2 c.c. of a very viscid dark bile-stained fluid, cloudy and containing a great deal of mucus. Microscopic examination showed large flakes of mucus, crystals of cholesterin and some bile pigment. By the direct microscopic method 83,000 bacterial cells per milligram of fluid were counted. Plate cultures on ascitic-fluid agar brought to development 175,000 and on litmus lactose agar 41,000 bacteria per c.c. of the fluid. Four colonies were transplanted from the plates of ascitic-fluid agar, and the cultures designated as Strains 103, 104, 105 and 106. Two taken from litmus lactose agar were preserved as 107 and 108. Fermentation tubes of glucose broth inoculated with 0.05 c.c. and 0.0002 c.c. of the fluid produced 60 per cent. and 40 per cent. gas, respectively, and tubes of lactose broth inoculated with similar quantities produced 75 per cent. and 60 per cent. gas, respectively. The Veillon tubes brought to development about 100,000 bacteria per c.c. of the fluid. Two of the colonies were transplanted and preserved as Strains 109 and 110. The tubes inoculated with spore material gave no growth.

*Fluid 32* was obtained from patient L. A., female, aged 38, suffering from an asthenic gastritis with indefinite pains in the stomach. The patient was convinced that she had a tapeworm but all examinations of the feces were negative. Gastric analysis December 3 showed total acidity 7, free hydrochloric acid 0; on December 5 total acidity 24, and free hydrochloric acid 16; on December 7 total acidity 8, and free hydrochloric acid 0. Examination of the urine was negative.

The duodenal tube was introduced at 10:30, November 29, to the distance of 95 cm. The patient fasted until 12:30 noon, November 30, when 2 c.c. of duodenal contents were aspirated.

The sample consisted of approximately 2 c.c. of golden-yellow fluid, quite clear. Microscopic examination was negative except for bacteria which numbered 6,800 per milligram of fluid, according to the direct microscopic count. Plate cultures inoculated with 0.05 c.c. and 0.0002 c.c. of fluid remained sterile, as also did the fermentation tubes and Veillon tubes inoculated with similar amounts.

*Fluid 33* was obtained from patient P. M., male, aged 45, suffering from alcoholic gastritis and convalescing from delirium tremens. No gastric or fecal analyses were made. Examination of the urine was negative.

The duodenal tube was introduced at 10:30 p. m., December 1, to the distance of 83 cm. The patient fasted until 9:15 a. m., December 2, when 3 c.c. of duodenal contents were aspirated.

The specimen consisted of approximately 3 c.c. of clear greenish-yellow fluid with a little mucus in the bottom of the tube. Microscopic examination showed crystals of cholesterin, mucus and unidentified debris, besides the bacteria, which numbered 22,000 per milligram of the fluid. Plate cultures on ascitic-fluid agar brought to development 1,800,000 and on litmus lactose agar 2,700,000 bacteria per c.c. of fluid. Two subcultures taken from the ascitic-fluid agar were preserved as Strains 111 and 112, and three from the litmus lactose agar as 113, 114 and 115. One of the usual set of fermentation tubes, namely the glucose broth inoculated with 0.1 c.c. of the fluid, produced a small bubble of gas; the others remained free from gas. The Veillon tubes developed numerous colonies which were not counted. Two of them were transplanted and the strains designated as 116 and 117. Tubes inoculated with spore material gave no growth.

*Fluid 34* was obtained from patient B. A., female, aged 30, suffering from myxedema and obesity. Analysis of the gastric juice December 14 showed total acidity 6, and free hydrochloric acid 0. The patient had about 30 pounds of superfluous fat. She complained of indefinite pains throughout the body. Examinations of the urine and feces were negative.

The duodenal tube was introduced at 7 a. m., December 9, to a distance of 116 cm. The patient fasted until 1 p. m., when 2 c.c. of duodenal contents were aspirated. This was used for bacteriological examination.

The specimen consisted of approximately 2 c.c. of a greenish-yellow fluid containing considerable mucus. Microscopic examination showed cholesterin crystals and 7,400 bacterial cells per milligram of the fluid. Plate cultures on ascitic-fluid agar brought to development 34,000 and on the litmus-lactose agar 21,000 bacteria per c.c. of the fluid. Strains 118, 119 and 120 were taken from ascitic-fluid agar and 121 and 122 from the litmus lactose agar. The usual set of fermentation tubes remained free from gas. The Veillon tubes brought to development a considerable number of colonies which were not counted. The examination of the spore material was omitted.

*Fluid 35* was obtained from the same patient B. A., as Specimen 34.

The duodenal tube was introduced at 7 a. m., December 11, to a distance of 109 cm. The patient fasted until 12:30 noon, when 10 c.c. of duodenal contents were aspirated. This was used for bacteriological study.

The specimen consisted of approximately 10 c.c. of a fairly clear golden-yellow fluid. Microscopic examination showed considerable unidentified debris, and the count showed 2,800 bacterial cells per milligram of the fluid. Plate cultures on ascitic-fluid agar brought to development 1,000,000 and on litmus lactose agar 620,000 bacteria per c.c. of the fluid. The usual set of fermentation tubes remained free from gas. The Veillon tubes brought to development numerous colonies which were not counted. The tubes inoculated with spore material remained sterile. Strains 123, 124, 125, 126 and 127 were taken from colonies on the ascitic-fluid agar and 128, 129 and 130 from the litmus lactose agar.

TABLE 1.—DATA CONCERNING THE FLUIDS EXAMINED

Fluid No.	Diagnosis	Age	Sex	Gastric Acidity		Tube Length Cm.	Date	Amount c.c.	Translucence	Color	Odor	Mucous	Food	Reaction to Litmus	Digestive State
				Total	Free HCl										
10	Neuritis .....	22	M	28	58	85	11/7	10	Cloudy	Lemon	0	0	+	Acid	Ewald
19	Neuritis .....	25	F	30	54	79	11/15	5	Cloudy	Golden	0	0	0	Neutral	Fasting
34	Myxedema and obesity	30	F	0	6	116	12/9	2	Cloudy	Lemon	0	Much	0	.....	Fasting
35	Same case as No. 34...	30	F	0	6	.....	12/11	10	Clear	Golden	0	0	0	Neutral	Fasting
29	Pyelitis (tuberculous)	23	M	.....	.....	90	11/25	3	Clear	Yellow	0	Viscid	0	Neutral	Fasting
18	Influenzal pneumonia..	24	M	.....	.....	63	11/14	3	Cloudy	Lemon	0	Flakes	0	Alkaline	Fasting
17	Intestinal tuberculosis.	43	M	20	35	86	11/14	3	Clear	Golden	0	0	0	Acid	Fasting
13	Cholecystitis .....	52	M	30	60	95	11/9	10	Clear	Lemon	0	0	0	Acid	Fasting
20	Typhoid relapse .....	18	M	.....	.....	91	11/15	2	Cloudy	Lemon	+	0	0	.....	Fasting
16	Prolapsed colon .....	24	F	15	50	.....	11/13	1	Clear	Golden	0	Slight	0	Alkaline	Fasting
24	Splanchnoptosis .....	40	F	13	27	77	11/18	3	Cloudy	Yellow	0	0	0	.....	Ewald
23	Splanchnoptosis .....	27	F	24	56	97	11/18	2	Clear	Golden	0	0	0	Neutral	Fasting
26	Asthenic gastritis .....	20	M	5	20	91	11/19	1	Cloudy	Golden	0	0	0	Acid	Fasting
28	Asthenic gastritis .....	29	M	0	12	91	11/21	4	Fluorescent	Golden	0	0	0	Neutral	Ewald
30	Asthenic gastritis .....	48	M	4	14	102	11/26	1	Clear	Golden	0	0	0	Neutral	Fasting
32	Asthenic gastritis .....	38	F	0	8	95	11/30	2	Clear	Golden	0	0	0	.....	Fasting
31	Gastric neurosis .....	48	F	13	32	80	11/29	2	Cloudy	Green	0	Much	0	.....	Fasting
33	Delirium tremens .....	45	M	.....	.....	83	12/2	3	Clear	Lemon	0	Slight	0	.....	Fasting
25	Cirrhosis of liver.....	42	M	.....	.....	88	11/19	23	Clear	Golden	0	0	0	Neutral	Fasting
27	Gastro-enterostomy ...	34	M	90	105	80	11/21	20	Clear	Golden	0	0	0	Alkaline	Ewald
15	Duodenal ulcer, healed (?) .....	34	M	20	89	83	11/12	3	Clear	Golden	0	Slight	0	Alkaline	Fasting
12	Duodenal ulcer .....	27	F	10	37	112	11/9	4	Clear	Orange	0	Slight	0	Alkaline	Fasting
11	Gastric ulcer .....	32	M	50	75	.....	11/7	7	Cloudy	Lemon	0	0	+	Acid	Ewald
14	Gastric ulcer .....	36	F	0	8	98	11/11	8	Cloudy	Yellow	0	Slight	0	Neutral	Fasting
22	Same case as No. 14...	36	F	0	8	.....	11/16	3	Clear	Golden	0	0	0	.....	Fasting
21	Gastric carcinoma*...	64	F	0	10	99	11/16	5	Cloudy	Golden	0	.....	0	Neutral	Fasting

\* The diagnosis was subsequently changed to atrophic gastritis in this case. † Cheesy. ‡ Much.

Another sample of duodenal fluid was obtained from this patient December 13. Chemical examination showed total acidity 5 (phenolphthalein), and the presence of amylase, lipase and trypsin.

The more important observations made in the series of examinations just described are summarized in Tables 1 and 2, in which the cases are grouped according to diagnosis, and in Table 3 in which the cases are rearranged in groups according to the number of colonies per cubic centimeter of fluid coming to development in the cultures.

TABLE 2.—DATA OF THE QUANTITATIVE BACTERIOLOGICAL EXAMINATIONS

Fluid No.	Direct Count Cells Per Cu. mm.	Cultures Inoculated with the Unheated Fluid*				
		Plate Cultures		Ascitic Glucose Agar. Tall Tubes Colonies Per c.c.	Fermentation Tubes	
		Litmus Lactose Agar Colonies Per c.c.	Ascitic-Fluid Agar Colonies Per c.c.		Dextrose Broth Gas Per Cent.	Lactose Broth Gas Per Cent.
10	132,000	0	10	0	0	0
19	367,000	0	0	0	0	0
34	7,400	21,000	34,000	.....	0	0
35	2,800	620,000	1,000,000	.....	0	0
29	114,000	80	1,600,000	1,000	0	0
18	125,000	2,300	3,400,000	†	‡	30
17	67,000	0	30	10	0	0
13	600	4	4	0	0	0
20	775,000	1,100,000	1,600,000	†	10	75
16	148,000	0	0	.....	0	0
23	124,000	150	550	200	0	0
24	496,000	80	130	50	0	0
26	864,000	150	850	350	0	0
28	30,000	50	120	150	5	75
30	33,000	2,400	2,600	10,000	‡	‡
32	6,800	0	0	0	0	0
31	83,000	41,000	175,000	100,000	50	68
33	22,000	2,700,000	1,800,000	†	‡	0
25	78,000	2,900,000	13,600,000	†	0	0
27	10,000	340	3,000	†	60	25
15	6,800	10	70	40	0	0
12	2,500	160	1,100	1,200	0	0
11	244,000	1,600	930	1,200	0	0
14	44,000	9,200,000	3,900,000	†	75	60
22	.....	31,000,000	85,000,000	†	50	.....
21	.....	1,600,000	1,000,000	†	30	.....

\* Tubes of ascitic-fluid agar and fermentation tubes of dextrose broth and lactose broth inoculated with spore material (i. e. the duodenal fluid heated at 80 C. for ten minutes), remained free from growth in every instance.

† Very many. ‡ Slight.



TABLE 3.—THE NUMBER OF CULTIVABLE MICRO-ORGANISMS PER CUBIC CENTIMETER OF FLUID

Fluid No.	Colonies Per C.C.	Gas in Ferment- tation Tubes	Dominant Types	Gastric Acidity		Diagnosis
				HCl	Total	
Cases Showing 0 to 100 Colonies Per c.c. of the Fluid.						
10	0-10	0	Coccus .....	28	58	Neuritis.
19	0	0	.....	30	54	Neuritis.
13	4	0	Coccus .....	30	60	Cholecystitis.
16	0-10	0	Bacillus .....	15	50	Prolapsed colon.
17	0-30	0	Coccus .....	20	35	Intestinal tuberculosis.
32	0	0	.....	0	8	Asthenic gastritis.
15	10-70	0	Coccus .....	20	89	Duodenal ulcer (healed ?).
Cases Showing 100 to 10,000 Colonies Per c.c. of the Fluid.						
26	850	0	Coccus .....	5	20	Asthenic gastritis.
23	550	0	Yeast .....	13	27	Splanchnoptosis.
24	130	0	Yeast, bacillus and coccus	24	56	Splanchnoptosis.
28	150	+	Coccus .....	0	12	Asthenic gastritis.
30	10,000	+	Coccus .....	4	14	Asthenic gastritis.
27	3,000	+	Yeast .....	90	105	Gastro-enterostomy, ulcer (?).
11	1,630	0	Coccus and bacillus.....	50	75	Gastric ulcer.
12	1,300	0	Coccus .....	10	37	Duodenal ulcer.
Cases Showing 10,000 to 1,000,000 Colonies Per c.c. of the Fluid.						
34	34,000	0	Coccus .....	0	6	Myxedema and obesity.
35	1,000,000	0	Bacillus .....	0	66	Same case as 34.
31	175,000	+	Bacillus .....	13	32	Gastric neurosis.
Cases Showing More Than 1,000,000 Colonies Per c.c. of the Fluid.						
18	3,400,000	+	Coccus and bacillus.....	.....	.....	Influenzal pneumonia.
29	1,600,000	0	Coccus .....	.....	.....	Tuberculous pyelitis.
20	1,600,000	+	Coccus and bacillus.....	.....	.....	Typhoid relapse.
33	1,800,000	+	Coccus .....	.....	.....	Delirium tremens, convalescent.
14	9,000,000	+	Bacillus .....	0	8	Gastric ulcer.
22	85,000,000	+	Bacillus .....	0	8	Same case as 14.
21	1,600,000	+	Coccus and bacillus.....	0	10	Gastric carcinoma.*
25	13,600,000	0	Coccus .....	.....	.....	Cirrhosis of liver.

\* The diagnosis of this case was subsequently changed to atrophic gastritis.

## NUMBER OF MICRO-ORGANISMS IN THE FLUIDS

*Direct Microscopic Count.*—The bacterial cells counted microscopically in the fluids varied from 600 to 860,000 per cubic millimeter, or from 600,000 to 860,000,000 per c.c. Evidently the bulk of these were actually dead; at any rate, the number of microbes brought to development in the cultures was only a small fraction of the number counted. These direct counts indicate that many bacterial cells gain entrance to the duodenum, doubtless after passing through the stomach, but otherwise they seem to have little or no significance.

*The Colony Count.*—The number of bacteria developing into colonies in cultures seems to bear a more definite relation to the gastro-intestinal condition. As will be seen in Table 3, those fluids showing less than 100 colonies per c.c., seven in number, were from cases showing, for the most part, normal gastric acidity. The free hydrochloric acid in these seven cases was 28-30-30-15-20-0-20 with an average of 20. The total acidity was 58-54-60-50-35-8-89 with an average of 51. The last two cases were somewhat abnormal and the findings in the sixth case are at least unusual when compared with the rest of this series.<sup>10</sup> Those fluids, from which between 100 and 10,000 colonies per c.c. were obtained, were derived from cases of gastro-intestinal disturbance. There were eight of these. The figures for free hydrochloric acid were, in order, 5-13-24-0-4-90-50-10, and for total acidity 20-27-56-12-14-105-75-37. There were three cases diagnosed asthenic gastritis, two as splanchnoptosis, one as gastric ulcer, one as duodenal ulcer and in one the operation of gastro-enterostomy had been performed. The three fluids, in which from 10,000 to 1,000,000 colonies per c.c. were obtained, were from cases diagnosed as myxedema with obesity, and gastric neurosis, respectively. In the former case the gastric juice showed free hydrochloric acid 0 and total acidity 6, and in the latter case hydrochloric acid 13 and total acidity 32. The fluids, from which a million or more colonies per c.c. developed, were from patients suffering from various diseases, most of them very ill. These results, as far as they go, would appear to indicate that normal duodenal fluid is practically free from living bacteria when food is absent, and that the number of colonies developed in cultures of the duodenal fluid is roughly an index of the digestive derangement. Where these are numerous the acidity of the gastric juice is often diminished, or there is other evidence of abnormality in the stomach or duodenum.

*Bacterial Spores.*—The cultures inoculated with spore material (the duodenal fluid heated to 80 F. for fifteen minutes) all failed to develop

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10. Three analyses of gastric juice from this patient gave the following results: December 3, total acidity, 7; free HCl, 0; December 5, total acidity, 24; free HCl, 16; December 7, total acidity, 8; free HCl, 0. The duodenal fluid was obtained November 30.

colonies. The result suggests that the passage of bacterial spores through the stomach into the duodenum is not ordinarily a prominent factor in the bacteriological relationships in the intestine.

*Gas-Forming Microbes.*—The direct inoculation of fermentation tubes of glucose broth and lactose broth with the duodenal fluid, with subsequent incubation at 37 C., detected gas-producing organisms in ten of the twenty-six fluids. These ten fluids were from nine different patients, suffering from influenzal pneumonia (1), typhoid fever (1), asthenic gastritis (2), gastric neurosis (1), delirium tremens (1), recurrent ulcer after gastro-enterostomy (1), gastric ulcer (1) and atrophic gastritis (1). It would seem that gas production in these cultures takes place only when there is considerable disturbance of digestion. In some instances the gas was evidently produced by bacilli and in other instances by yeasts.

#### GENERAL NATURE OF THE MICRO-ORGANISMS ISOLATED

During the progress of the work, 130 colonies were transplanted and preserved for later study. Of these, twenty-two strains were lost before being studied; two others, Strains 21 and 34, were discarded because they were derived from fluids (No. 16 and No. 19) which gave rise to so few colonies that these were regarded as contaminations. Four of the strains derived from Case 20, those designated as D 20 (1), D 20 (2), D 20 (3) and D 20 (4), were identified as *B. typhosus* by their morphology, cultural characters and agglutination by a typhoid serum. The remaining 106 strains have been studied and grouped according to their morphology, reaction to the Gram stain, gas production in glucose broth and liquefaction of gelatin. The groups thus established are composed of various kinds of bacteria, but serve roughly to classify the organisms.

*Gram-Positive, Non-Liquefying Cocci.*—Forty-three of the 106 strains belong to this group, and organisms of this type were isolated from sixteen of the twenty-six fluids examined—fluids 12-13-15-17-18-20-21-23-24-25-26-28-29-31-33 and 34. The fluids from which organisms of this group were not isolated were ten in number, namely, 10-11-14-16-19-22-27-30-32-35. Four of these, 10-16-19 and 32, were approximately sterile fluids, and in the case of Fluid 11, only one subculture survived for study. Three of the remaining five fluids contained more than 500,000 cultivable micro-organisms per cubic centimeter, and the cocci of this group may have been easily missed though present. In the other two instances there were about 3,000 colonies developed per cubic centimeter of fluid. The strains transplanted, four in one instance and five in the other, did not include an organism of this group.

The fluids from which Gram-positive and non-liquefying cocci were isolated included two which were nearly sterile, namely, 13 and 17, and

this was the only type of microbe found in No. 13. In those cases in which the colonies numbered between 40 and 1,000 per cubic centimeter, organisms of this type constituted the bulk of the living flora. Here were included one case of healed duodenal ulcer, No. 15, two cases of splachnoptosis, Nos. 23 and 24, two cases of asthenic gastritis, Nos. 26 and 28, and one case of renal tuberculosis. In one fluid, which produced about 20,000 colonies per c.c., No. 34, these cocci were dominant, as was also the case in one fluid showing 2,000,000 colonies per c.c., a convalescent case of delirium tremens, and in another showing 3,000,000 colonies per c.c., a case of advanced cirrhosis of the liver. In other instances in which abundant colonies developed, the dominant organism was something else, even though these cocci were found. These instances included one case of influenzal pneumonia, one of typhoid fever, one of gastric neurosis and one of asthenic gastritis. In the cases of ulcer these cocci were also forced into the back-ground by the dominance of other types. It would seem that bacteria of this group appear in the duodenal fluid in slight disturbances and are also present in more grave conditions, although they may be obscured by excess of other microbial types.

*Gram-Negative, Gas-Forming Bacilli, which Liquefy Gelatin.*—Nineteen of the 106 strains belong in this group. They were isolated from only five of the twenty-six fluids examined, and two of these were derived from one individual. A single strain of this type was isolated from the case of influenzal pneumonia, No. 18, in which the fluid produced 100,000 colonies per c.c. In the case of typhoid relapse, No. 20, two of the twelve strains isolated belonged to this group. One case, No. 21, diagnosed at the time as gastric carcinoma, seemed to have more living bacteria of this type than any other. Three of the five strains isolated from this fluid belong here. In this instance, 1,500,000 bacteria per c.c. of the fluid developed into colonies. The fourth case was diagnosed as gastric ulcer and the diagnosis confirmed at operation. Two fluids, No. 14 and No. 22, were obtained from this case November 11 and November 16. In the former 4,000,000 and in the latter 30,000,000 bacteria per c.c. developed into colonies, and these Gram-negative liquefying bacilli were the only kind represented in the thirteen subcultures. It would appear, therefore, that the bacteria of this type occur only in severe or very severe gastro-duodenal disturbance. The group may, perhaps, represent a single species. It is of unusual interest because of the fermentative properties, somewhat unusual for intestinal bacteria, and because of its abundance in some of the more severely ill patients.

*Liquefying Gram-Positive Cocci.*—Thirteen of the 106 strains belong in this group. Some of them were staphylococci and some appeared to be sarcines. They were found in eight of the twenty-four individuals. In only one case were they very numerous, and this was the case of



influenzal pneumonia, No. 18, in which the fluid produced 100,000 colonies per c.c., and three of the six strains isolated were found to be liquefying staphylococci. The remaining strains of this group were isolated from Fluid 10, which was almost sterile; No. 15, which produced forty colonies per c.c.; No. 12 and No. 30, which produced 1,100 and 2,500, and Fluids 25-31-34 and 35, which produced millions of colonies per c.c. These bacteria would appear, therefore, to be present in a very wide range of conditions, though probably not so commonly present as the cocci of the first group.

*Yeasts.*—Twelve of the 106 strains were cultures of yeast, and these were derived from five of the twenty-four patients examined. Fluids 20, 24 and 35 each furnished one strain of yeast. Two cases, No. 23 and No. 27, furnished all the rest. Fluid 23 was taken from a case diagnosed splachnoptosis, and it produced 300 colonies per c.c. of fluid. Five of the nine strains isolated proved to be yeasts. Case 27 was one in which a gastro-enterostomy had been performed for the cure of ulcer. The fluid produced 4,000 colonies per c.c., and all four of the strains isolated proved to be yeasts.

*Pleomorphic Bacteria.*—Six of the 106 strains were made up of branched threads and spherical elements. These were at first regarded as mixed cultures, but plating failed to separate them, and it seems probable that the various forms present may belong to the same species. These forms occurred in only two specimens, No. 30 and No. 31. They constituted the dominant type in No. 30, in which 2,500 colonies developed per c.c. of fluid. This was a case of asthenic gastritis. In No. 31 only two of the eight strains isolated belong in this group. This case was diagnosed as gastric neurosis and 100,000 bacteria per c.c. of fluid developed in the cultures.

*Gram-Positive Strepto-Bacilli.*—Five of the 106 strains were made up of very regular bacilli joined together in long threads. Four of these failed to liquefy gelatin. Of these, two were isolated from Fluid 23 and two from Fluid 24, both diagnosed as splachnoptosis, the number of colonies produced numbering 300 and 100 per c.c., respectively. The fifth strain in this group resembled the others in form and staining properties, but it caused liquefaction of gelatin. This was isolated from Fluid 31, from the case diagnosed as gastric neurosis.

*Irregular Bacilli.*—Bacilli irregular in shape and also in their reaction to the Gram stain are represented by four of the 106 culture strains. One of these was derived from Fluid 11, diagnosis of the case being gastric ulcer, and three were from Fluid 35, furnished by an individual suffering from myxedema and obesity.

*Gram-Negative Gas-Forming Bacilli, which do not Liquefy Gelatin.*—Only three of the 106 strains belong here. One was isolated from

Fluid 18, the case of influenzal pneumonia, and the other two from No. 31, a case of gastric neurosis. In each instance the cultures showed about 100,000 colonies per c.c. of fluid, and the colonies of this particular type were in the minority. This is, perhaps, one of the surprising features of this study, as it might have been expected that organisms of the *B. coli* group would be somewhat prominent members of the duodenal flora, in some conditions at least.

*Gram-Negative Bacillus Producing Yellow Pigment.*—One strain, isolated from Fluid 34 (case of myxedema and obesity), was a Gram-negative bacillus, morphologically like *B. coli*, which does not liquefy gelatin nor produce gas from dextrose, but does form a yellow pigment.

#### SUMMARY

1. It is possible, with proper care, to obtain a sample of the intestinal juice through the Einhorn duodenal tube sufficiently free from contamination for bacteriological study.

2. The normal duodenal fluid during a fast is almost free from living micro-organisms, although numerous bacterial cells are always visible on microscopic examination. The few living microbes obtained in cultures from such fluids are generally Gram-positive cocci.

3. In various gastro-intestinal disturbances, the number of cultivable microbes in the duodenal fluid is markedly increased. These organisms are of several different varieties, bacilli, cocci, yeasts and branching thread forms being represented in different cases.

4. In the one case of typhoid fever examined, *B. typhosus* was isolated from the duodenal fluid.

5. The bacteriological study of intestinal juice obtained in this way would seem worth while in cases of achylia gastrica with diarrhea and in cholecystitis. It also seems to us to be a promising field for investigation in those obscure diseases, the causation of which is sometimes ascribed to abnormal intestinal digestion.

6. It may also, perhaps, prove to be a procedure of value in the early diagnosis of typhoid fever, and in the detection of typhoid carries. We think it should be given a trial in this disease, as suggested by the previous work of Hess.

Acknowledgments are due to Miss Laura M. Stryker for assistance in the laborious microscopic and cultural examinations; to Dr. B. Lattin and Dr. W. G. Lough for personal attention to details in collecting the specimens from the patients, and to Prof. V. C. Myers for the data concerning the chemical examination of the duodenal fluids.

We also desire to express our obligation and thanks to Dr. Edward Quintard, Director of the Department of Medicine, for permission to use the clinical material.

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# THE FREQUENCY OF LOW POLYMORPHONUCLEAR LEUKOCYTE WITH HIGH LYMPHOCYTIC DIFFERENTIAL COUNTS \*

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In the course of routine blood examinations made in Lane Hospital, San Francisco, it had been frequently noted that the polymorphonuclear leukocytes were below 60 per cent. in apparently normal individuals, and also that the lymphocytes ran over 40 per cent. in a sufficient number of apparently normal persons to make a differential count of somewhat doubtful value in the diagnosis of exophthalmic goiter, etc. In order to determine, if possible, the cause of these variations from the commonly accepted standard, the blood of one hundred normal individuals was examined.

These were selected with a view to including outdoor as well as indoor workers, females as well as males, and ages between 21 and 50; in short, to approximate as nearly as possible, the average individual appearing in the clinics or hospital. Among those examined were physicians, students and nurses at Lane Hospital, soldiers at the Presidio and such miscellaneous people as could be procured from time to time. All were in their usual state of health, care being taken to exclude those having recent illness. No other examination was made.

Various methods of spreading the blood were used. Ehrlich's method with coverslips, Da Costa's method with slides and Craig's methods with cigarette papers were tried and gave practically the same results. Two slides were made from each individual, two hundred cells being counted from each slide with an additional hundred in doubtful cases. Wright's stain was used. A total leukocyte count unfortunately could be made in only twenty of the hundred cases.

Average leucocytic count (20 cases) was 7,000.

Average differential count (100 cases)	Per cent.
Polymorphonuclear Neutrophils .....	56.55
Lymphocytes .....	37.45
Large Mononuclears and Transitionals.....	4.51
Eosinophils .....	.97
Basophils .....	.52
Polymorphonuclears in classes:	
(35) Soldiers .....	58.28
(22) Nurses .....	49.42
(18) Doctors and students .....	57.88
(25) Miscellaneous .....	58.96

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\* Submitted for publication May 31, 1913.

\* From the Clinical Laboratory of Lane Hospital, Stanford University Medical Department.

Looking at these figures from another standpoint:

The polymorphonuclears in 93 of the total number were under 70 per cent.  
 The polymorphonuclears in 86 of the total number were under 65 per cent.  
 The polymorphonuclears in 59 of the total number were under 60 per cent.  
 The polymorphonuclears in 37 of the total number were under 55 per cent.  
 The polymorphonuclears in 22 of the total number were under 50 per cent.  
 The polymorphonuclears in 10 of the total number were under 45 per cent.

These results are markedly lower than those given in the text-books.

	Neutros. Per Cent.	Eos. Per Cent.	Basophils Per Cent.	Small Monos. Per Cent.	Large Monos. Per Cent.	Transitionals Per Cent.
Ehrlich and Lazarus (in Nothnagel). . . . .	70-72	2-4	.5	22-25	1	2-4
Naegeli (1912) . . . . .	65-70	2-4	.5	25		
Pappenheim (1911) . . .	73-75			20-22	2-8	
Krehl (1907) . . . . .	70	1-4		28		
Cabot (in Osler) . . . . .	60-70	.5-4	.1-.5	20-40 of which		1-10
Sahli (1911) . . . . .	70-72	2-4	.5	22-25		2-4
Howell (1912) . . . . .	60-75	2	.5	20-25	1	2-10
Da Costa . . . . .	60-75	.5-5	.5	20-30	4-8	

On looking into the literature, among others who record low polynuclear counts, I find that Bunting<sup>1</sup> (1911) in Wisconsin got a neutrophil average of 54.6 per cent., large and small lymphocytes 35 per cent., transitionals 7.4 per cent. He believes that geographical location and altitude have some bearing on his results.

Guerrero and Sevilla,<sup>2</sup> as well as Chamberlain,<sup>3</sup> all work in the Philippines. Guerrero and Sevilla found polynuclears, 51 per cent., large and small 38.5 per cent.; Chamberlain, polynuclear average 54 per cent. Both believed that the tropical climate had an influence on the result.

Watkins,<sup>4</sup> in Arizona, says, in summing up the results of his counts:

There is present in many individuals a considerable increase in the percentage of lymphocytes so that instead of being 30 per cent. they approximate 40, 45 or 50 per cent., or more. These increases are noted where the atmosphere is rarified by absence of moisture and by high temperature.

Hoxie,<sup>5</sup> in Missouri, gives low polymorphonuclear counts ranging from 67 to 49 per cent., which he attributes to auto-intoxication due to colonic stasis.

1. Bunting: Am. Jour. Med. Sc., 1911, cxlii, 698.

2. Guerrero and Sevilla: Philippine Jour. Sc., Series B, 1909, p. 277.

3. Chamberlain: Philippine Jour. Sc., 1911, vi, 441, Series B.

4. Watkins, W.: Jour. Am. Med. Assn., 1911, lvii, 2129.

5. Hoxie: Jour. Am. Med. Assn., 1912, lviii, 1493.



Watkins (in same paper) gives counts on cured tuberculous patients with lymphocytes ranging from 40 to 70 per cent. Looked at from these standpoints, our results may have been influenced by

- (a) Climate and geographical location.
- (b) Previous tuberculous infections (I have no data on this matter).
- (c) Auto-intoxication (extremely likely in a number of cases).
- (d) The number of individuals examined is hardly large enough to eliminate chance.

To get a general idea of the range of the differential counts as they ordinarily present themselves in adults, the results of 500 counts were analyzed. These were taken from the medical, neurological, gynecological and surgical clinics and corresponding services at Lane Hospital—active tuberculous cases not being treated at either. The counts were made by Dr. Mylott of the Clinical Laboratory and by the intern staff (eight) of Lane Hospital, necessarily giving a more average technic.

Only those differential counts were included whose total leukocyte count was 10,000 or below.

The polymorphonuclears in 87 per cent. of the 500 were under 70 per cent.  
The polymorphonuclears in 69 per cent. of the 500 were under 65 per cent.  
The polymorphonuclears in 46 per cent. of the 500 were under 60 per cent.  
The polymorphonuclears in 30 per cent. of the 500 were under 55 per cent.  
The polymorphonuclears in 15 per cent. of the 500 were under 50 per cent.  
The polymorphonuclears in 2 per cent. of the 500 were under 45 per cent.

These counts correspond fairly well with the results observed in supposedly normal people; considerably lower than the accepted normal. In fact, 75 per cent. "polymorphs" was so seldom reached that we were taught almost invariably to expect a numerical count greater than 10,000 when that number was obtained.

#### CONCLUSIONS

Our results and those reported in the literature would seem to indicate that low polymorphonuclear differential counts are very frequently observed in clinical work. Whether due to climate, geographical location, previous disease, condition of metabolism, or combination of these factors is not clear. At any rate, it becomes of importance for the clinician to remember the existence of this condition when he is tempted to give undue significance to a high lymphocytic count.

I am indebted to Dr. T. Addis for helpful suggestions, and to Capt. Hopwood, U. S. Medical Corps, for his courtesy in obtaining smears.

# A CASE OF ACROMEGALY AND POLYGLANDULAR SYNDROME, WITH SPECIAL REFERENCE TO THE PINEAL GLAND\*

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The term polyglandular syndrome is used by most writers to express the disturbed function of two or more glands of internal secretion. Cushing<sup>1</sup> has confined the term to cases in which it is impossible to tell which of the structures is primarily at fault. The combinations of the different glands is variable, as is the degree to which each is changed. From experimental data it is seen that the absence of the function of a normal gland (as for example, extirpation of that gland) leads to a definite symptom-complex in which some of the other glands participate. For instance, in the preadolescent eunuch, the impotency and the undeveloped secondary sexual characteristics are due to the immediate effects of the castration, while the growth of the long bones is thought to be due to a secondary hyperplasia of the anterior lobe of the hypophysis. Fischera<sup>2</sup> and others, have definitely shown that a secondary hyperplasia of the glandular part of the hypophysis, follows castration. In addition to the hypophyseal changes, Calzolarie, in 1898, and Henderson,<sup>3</sup> in 1904, respectively, have shown that in castrated rabbits and cattle the thymus may undergo hypertrophy and its involution be delayed.

The disturbance of function of one of the internal secreting glands also leads to a polyglandular involvement, as for example in exophthalmic goiter. In 135 cases of Graves' disease collected from the literature by Matti,<sup>4</sup> 74 per cent. were associated with a persistent thymus, while out of sixty cases reviewed by Capelle,<sup>5</sup> the thymus was enlarged in 79 per cent. of the cases. Since the symptom-complex of exophthalmic goiter has not been reproduced in animals, it is thought to be due to a perverted secretion of the thyroid gland. Just what the biologic disturbance is that underlies this abnormal secretion, has not been discovered.

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\* Submitted for publication June 4, 1913.

\* From the Department of Pathology, University of Chicago.

1. Cushing, H.: "The Pituitary Body and Its Disorders," 1912, p. 213.

2. Fischera, G.: "Ipofisi e Castrazione," Policlinico, Rome, 1910, xvii, 333.

3. Quoted by C. A. Parker: Surgery of the Thymus Gland, Am. Jour. Dis. Child., 1913, v, 89.

4. Matti, quoted by A. Crotti: Thymus Tracheostenosis and Thymus Death, Jour. Am. Med. Assn., 1913, lx, 571.

5. Capelle: Die Beziehungen der Thymus zum morbus Basedowii. Beitr. z klin. Chir., 1908, lviii, 353.

There are many other well known polyglandular syndromes, depending on either the ablation, the hyposecretion, the hypersecretion or the perverted secretion of certain glands. Prominent among these is acromegaly, supposedly due to a hyperplasia of the chromophil cells of the anterior lobe of the hypophysis. Acromegaly, or adult gigantism,<sup>6</sup> has never been reproduced in animals. It is accompanied by a polyglandular syndrome, involving one or more of the glands of internal secretion besides the hypophysis, which is always involved.

When in 1888, Marie<sup>7</sup> first pointed out the connection between hypophyseal strumas and acromegaly, he thought that a hyposecretion of the hypophysis was the probable cause; since then he has come to the conclusion that acromegaly is a perverted secretion of the anterior lobe. Others, namely, Silvestrini in 1894, Arnold in 1894, Petren in 1907, and Warda in 1901, have maintained that acromegaly and hypophyseal lesions are associated only accidentally, because they and others have performed autopsies on individuals dying from acromegaly in whom no pituitary lesion was found. These cases, however, must be accepted with reservation, for it is known that accessory nodules of hypophyseal tissue simulating anterior lobe tissue, may be found within the body of the sphenoid bone,<sup>8</sup> in the roof of the pharynx, and anywhere along the fetal craniopharyngeal duct. Lewis<sup>9</sup> reported a case of incipient acromegaly, in which the hypophysis was of normal size, yet presented histologically a proliferation of the chromophil cells of the anterior lobe.

Another theory of acromegaly points to an underlying biologic disturbance as the cause; this theory considers that the enlargement of the hypophysis is only one feature of a polyglandular syndrome, and that it is the result instead of the cause of acromegaly. Although no one disputes that some unknown disturbance underlies the gradual hypertrophy of the anterior lobe in acromegaly, clinical and anatomical evidence support the fact that bone changes follow instead of precede the hypophyseal hyperplasia.

Mussalango (1892), Tamburni (1894), Benda (1901), Modena (1903) and Fischer (1910), consider acromegaly the result of an hyperplasia or adenomatous condition of the anterior lobe of the hypophysis. An adenomatous struma is the type of tumor usually found in acromegalic individuals who come to autopsy.

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6. Cushing, H.: Quoting (a) Brissaud, E., and Meig, H.: *Rev. scientif.*, 1895, iii, 330, and (b) Lannois, P. E., and Roy, P.: *Etude biologique sur less geants*, Paris, 1904. See note 1.

7. Marie, P.: *L'Acromegalic*, *Nouv. Icon de la Salpêtrière*, 1888, i, 173.

8. Erdheim, J.: *Übereinen Hypophysentumor von ungewöhnlichen Sitz.*, *Beitr. z. path. Anat.*, 1909, xlv, 233.

9. Lewis, D.: *Hyperplasia of the Chromophil Cells of the Anterior Lobe of the Hypophysis as the Cause of Acromegaly*, *Bull. Johns Hop. Hosp.*, 1905, xvi, No. 170.

From birth on, the chromophil cells of the anterior lobe of the hypophysis increase; at 40 they are equal in number to the chromophobe cells of the same lobe; from 40 on the chromophil cells diminish in number so that in old age the chromophobes are again in preponderance as they are at birth (Lewis). During puberty, pregnancy and the climacteric, physiological hyperplasia of the chromophil cells is apparent. During the course of an acute disease, young patients often grow quite noticeably, a fact perhaps attributable to hyperplasia of the anterior lobe. Further, after castration, thyroidectomy and after pancreatectomy, the anterior lobe is said to undergo enlargement. Benda,<sup>10</sup> in 1900, and Lewis,<sup>9</sup> in 1905, were the first to emphasize the fact that in the early stages of acromegaly, there is an hyperplasia of the chromophil cells of the anterior lobe, which apparently is the cause of the bone changes. Cushing, Fischer and others have adopted this view. Most investigators consider the chromophil cell as the most active cell in the gland. From clinical, anatomical and experimental findings, one is justified in assuming that these cells govern bone growth to a large extent. Massalongo and others have shown that if hypersecretion of the anterior lobe occurs in the early part of life before ossification of the long bones is completed, gigantism results; if, after ossification of the epiphyses, hyperplasia of the chromophil cells occurs, acromegaly results. In other words, acromegaly is adult gigantism.

It must be remembered that the chromophil cells composing hypophysial strumas, which are the cause of acromegaly, undergo degenerative processes early, in so far as the acidophil granules of these cells disappear entirely or in part. Small regions of necrosis occur, and cyst-like formations appear. It is probable that the so-called chromophobe strumas described in cases of acromegaly, as in most of Cushing's cases, are chromophil cell tumors from which the acidophil granules have disappeared through degenerative processes.

Assuming that increased secretion of the anterior lobe of the hypophysis is the cause of acromegaly and gigantism, one might suppose that feeding and injection experiments would produce overgrowth in puppies, while in old dogs some acral change would result. Neither of these results has been obtained. Caselli,<sup>11</sup> Cushing,<sup>12</sup> Aldrich,<sup>13</sup> Miller<sup>14</sup> and

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10. Benda, C.: Beiträge zur normalen und pathologischen Histologie der menschlichen Hypophysis cerebri., Berl. klin. Wchnschr., 1900, 37, 1205.

11. Caselli, A.: Influence de la fonction de l'hypophysis sur le developpment de l'organisme, Riv. sper. di frenatria, 1900, xxxvii, 176.

12. Cushing, H.: The Pituitary Body and Its Disorders, 1912, p. 11.

13. Aldrich, F. B.: The Feeding of Young White Rats with Dessicated Anterior and Posterior Lobes of the Hypophysis Separately, Am. Jour. Physiol., 1912, xxxi, 94.

14. Miller, J. L.: Quoted by Aldrich. See note 13.



others, find that injection and feeding experiments not only do not increase the stature of animals, but retard their growth and stunt them; Caselli and Cushing worked with dogs, while Aldrich and Miller worked with white rats. Parisot<sup>15</sup> also found no noticeable changes with repeated injections of anterior lobe extracts into adult dogs, nor in puppies injected with small doses. With large injections in young animals, diminution in weight and size resulted. These findings suggest that the secretion of the chromophil cells of the anterior lobe is altered or perverted in acromegaly and gigantism, but by no means prove it, since injection and feeding of the gland or its extracts is crude and unreliable in comparison to the well-balanced mechanism of internal secretion.

Hyposecretion of the anterior lobe of the hypophysis is accompanied by bone changes exactly opposite to those of hypersecretion. It is generally known that young women suffering from exophthalmic goiter have delicate features and a small bony frame, as compared to the coarse features and large bones of the acromegalic or giant. Lewis and Benda both found a decrease in the eosinophilic type of chromophil cells of the anterior lobe of the hypophysis in patients dying from exophthalmic goiter.

The fact that heteroplastic tumors of the anterior lobe of the hypophysis occur without acromegaly or gigantism, is in accord with the findings of Benda, Lewis, Cushing, Fischer and others, relating to hyperplasia of the chromophil cells. Parisot<sup>15</sup> collected from the literature reports of seven cases of tuberculosis and of eight cases of gumma of the hypophysis, none of which had symptoms of acromegaly or gigantism. Many cases of sarcoma and of adenosarcoma accompanied by acromegaly have been reported in the literature, but in view of our present knowledge concerning the similarity of chromophil and of chromophobe strumas to sarcomas, reconsideration of the actual classification of these tumors must be made. Chromophil strumas and chromophobe strumas differ from sarcoma elsewhere, first, in so far as their course is longer, the average being three years; second, because they do not produce metastasis nor infiltrate the surrounding tissue, and third, as Mitchell and Le Count<sup>16</sup> have pointed out, the process of cell division is far less frequent than in sarcomas. In the case reported here, mitotic figures are extremely infrequent.

The following case of polyglandular syndrome was in a patient in the service of Dr. C. H. Lovewell of this city, through whose kindness and

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15. Parisot, J.: *Le rôle de la hypophysis dans la pathogenie de l'acromegalie*, *Rev. Neurologique*, 1910, xix, 277.

16. Mitchell and LeCount, E. R.: *Report of a Necropsy in a Case of Acromegaly with a Critical Review of the Recorded Pathological Anatomy*. New York Med. Jour., 1899, lxix, 517.

efforts an autopsy was permitted. The autopsy was performed by Dr. H. Gideon Wells and myself, on Nov. 20, 1912, about eight hours after the death of the patient.

#### CASE REPORT

*Clinical History.*—The patient died Nov. 19, 1912, at the age of 25. The parents state that he had moments of moodiness and forgetfulness when a boy. About one year before his death he noticed that his hands were getting larger; at this time he had occasional slight headaches. Two years prior to this, during his married life, he had been extremely passionate. About six months before his death he began to lose sexual desire, and two months later or four months before his death he became impotent. Mental symptoms of moodiness, forgetfulness and irritableness were becoming evident to his wife and also to the man for whom he worked as a driver. At about this time polyuria was marked; diaphoresis was so intense that during the night he would soak all of the bed clothes. One month before death headaches were frequent and very severe; his eyesight was becoming foggy, especially that of the left eye. November 13 he had a frightful headache and had to quit work. He was conscious until noon of November 18; he died at 4 a. m., November 19. It is thought by his wife that he became a little heavier during the few months prior to his death.

*Autopsy Report.*—The body is that of a man 6 feet and 3 inches long, well muscled and large boned. The weight is approximately 160 pounds; there is no abnormal amount of panniculus. The texture of the skin is normal. The face is large considered in its entirety, but the cheek bones, superciliary ridges, nose, and jaw, although of good size, are not unduly prominent. Nothing abnormal can be seen or felt about the head except that the right pupil is larger than the left one. The chest is a little flattened. The fingers and thumbs are noticeably enlarged, with the thickening which seems to be fibrous, more transverse than antero-posterior. The left forefinger measures 75 mm. about the middle joint which is 10 mm. more than normal. The second joint of the second finger of the same hand measures 74 mm., which again is about 7 mm. more than normal. The corresponding joint of the middle finger of the right hand is 71 mm. in circumference. Both hands are perceptibly broadened. Each hand around the knuckles measures 211 mm. There is no apparent enlargement of the feet. The toes are in extreme extension. The superficial lymph-nodes are all slightly enlarged. The costochondral junctions are not swollen.

The abdominal cavity is free from fluid. There are a few fibrous adhesions about the gall-bladder and the spleen. The fat in the omentum, as elsewhere, is scanty. The liver extends three fingerbreadths below the costal margin on the right side. The retroperitoneal lymph-nodes and those in the mesentery are markedly enlarged; the largest mesenteric lymph-node measures 20 x 15 x 6 mm. Those lymph-nodes about the pancreas are even larger. The pleural and pericardial cavities are normal. The lymph-nodes in the mediastinal and in the cervical regions are enlarged and quite firm; on sectioning, their surfaces are reddish-gray in color. Nothing of importance is found in the trachea, lungs, esophagus or stomach. The intestines are full of fluid; the solitary follicles and Peyer's patches are enlarged.

The thyroid gland is about twice its normal size, weighing 65 grams, and contains a large amount of colloid evenly distributed throughout. An accessory lobule, 12 x 15 x 10 mm. is found behind and attached closely to the right lobe.

The thymus is persistent, well defined from the surrounding tissue and consists of two lobes, the right one of which extends in a finger-like projection up to the region of the thyroid. Its weight is 22 grams and its total length is 85 mm. Its color is pinkish, while its consistence and cut surface appearance is that of a thymus of a normal infant. Three small accessory thymi are found distributed among the cervical lymph-nodes, the highest point reached by any of them being

the lower pole of the thyroid; the largest of these accessory glands is 15 mm. in diameter, while that of the smallest is 5 mm.

The heart and its valves are normal in size and in appearance. The left ventricle is in firm systole. The aorta is elastic and its intima is smooth.

The liver weighs 2,100 grams; it is darker and softer than normal, and its cut surface bulges slightly from beneath the capsule.

The spleen is enlarged, weighing approximately 360 grams. It is a trifle soft. The cut surface is not abnormal in appearance except that the Malpighian bodies are inconspicuous.

The adrenals are of normal size and appearance. The left one weighs 5.5 grams. Both kidneys are considerably larger than normal. The left one weighs 161 grams. Their consistence is rather soft; the blood vessels are dilated. The cut surface is swollen; the cortex is mottled while the pyramids are pale.

The prostate, urinary bladder and ureters are normal. Unfortunately the testicles were not obtained. The external genitalia are well developed.

On opening the skull the calvarium is found to be very soft and thin. In places it is not over 1.5 mm. in thickness. The sutures of the skull are still distinct. There is a very little cerebrospinal fluid. The cerebral convolutions are broad and flat while the sulci are shallow and narrow. The brain is quite soft and dry; the lateral ventricles contain less fluid than normal. The sella turcica is enlarged so that it is 2 cm. deep and 2.5 cm. wide. It is tensely filled by the hypophyseal tumor, which has extended upward and backward into the third ventricle, distorting and partly destroying the infundibulum. Upward and forward it comes in contact with the posterior surface of the optic chiasma, which it has flattened out considerably. A fairly large flat tumor mass of rusty brown color and of a coarsely granular, spongy nature, extends anteriorly on the body of sphenoid bone, just posterior to the cribriform plate, which is intact. The bony wall of the sella turcica is thinned out from pressure of the tumor, but it is not invaded or broken through by the tumor mass.

Very conspicuous is the pineal gland which protrudes 5 mm. beyond the posterior edge of the corpus callosum. It is found to be 13 x 5 x 6 mm. in its various diameters, the antero-posterior diameter being the largest; its weight is 940 mg.

*Microscopical Examination.*—The alveoli of the thyroid gland are full of colloid, with an abundant number of desquamated parenchymatous cells.

The thymus gland resembles in all respects that of an infant; the capsule is thin and the lymphoid tissue more abundant at the periphery of the lobules than at the centers where the large lymphoid cells predominate. Hassall's corpuscles are numerous and large, their centers are often cyst-like and full of fatty cellular debris. The arterial walls are not thickened as in a thymus of a normal individual of this age, nor is there any fatty infiltration or perilobular fibrosis indicative of involution. The two accessory thymus nodules are of a similar structure.

All the lymph-nodes examined, including the mesenteric, the peribronchial and the cervical, represent a stage of active proliferation of the endothelial cells in the germinal centers as well as in the stroma. These endothelial cells are so numerous that the glands take a general pink stain with eosin.

The chromaffin cells of the adrenal have increased in size and in number, extending in some places through the cortical cell layer almost to the capsule.

In the spleen a condition of endothelial cell hyperplasia, similar to the condition in the lymph-nodes, is found.

Both the liver and the kidney present a slight connective tissue hyperplasia, and to some extent a swelling and granular condition of the parenchyma.

The heart muscle is of normal appearance.

The parathyroid glands are slightly larger than normal. The nuclei here, as in the adrenal medulla, take a deep stain with hematoxylin, indicating an abundance of chromatin material. A great many of the parenchymatous cells



here are vacuolated, while the cytoplasm of other remaining cells is solid, homogeneous, and deep pink staining with eosin. Colloid material is very scant.

The pineal gland is oval, being 13 mm. long and 6 mm. in depth. About the entire periphery of a sagittal section through the center of the gland there is a border of deeper staining tissue which is composed of cells with a diffuse, poorly outlined, and light pink staining cytoplasm, with large oval or pear-shaped vesicular nuclei; these nuclei average 13 microns in diameter but often reach 20 microns. The nucleolus is not prominent, at least not definitely distinguishable from small round masses of chromatin. Connective tissue trabeculae traverse this cellular border, extending from the thin capsule radially towards the center of the gland, dividing the cellular periphery into numerous compartments. Capillaries are fairly numerous and course mainly in the connective tissue trabeculae. The arrangement of the cellular border is, on the whole, in irregular cords and masses, and in ill defined alveolar structure, simulating tubules with an indefinite lumen of small caliber. At the tail end of the body, the parenchymatous layer is 3 mm. thick; the connective tissue trabeculae in this region are occasionally 30 microns thick and are quite vascular. No pigment (melanin) or any granules of a secretory nature can be seen. Psammomata are lodged in small groups, especially at the inner edge of the cellular border; they are not numerous. The evaginated pineal recess penetrates one-third the length of the gland; it is duct-like and is lined with low cuboidal epithelium. Intermixed with the parenchymatous cells of the border, are the small round and deep staining glial cell nuclei.

The carotid gland is of normal size and consists of small masses of chromaffin cells surrounded by thick connective tissue whorls.

The hypophyseal tumor is composed of masses of oval or round cells, with a fairly well defined cytoplasmic border. The nuclei are either oval or round and are deeply stained with hematoxylin; they average 7 microns in diameter. There is no set arrangement of these tumor cells although they assume an indefinite alveolar structure in places. Mitotic figures are extremely infrequent. In many instances two nuclei are found in one cell. With Van Gieson's stain there are seen delicate strands of connective tissue here and there in which run capillaries; compared to the normal hypophysis, the blood-supply and connective tissue are very scant. The tumor cells lie directly on the endothelium of the blood vessels as they do in sarcoma. The tumor mass is the seat of numerous diffuse hemorrhages and also regions of necrosis ranging from 30 microns to 2 mm. in diameter. The origin and nature of the cells composing this tumor are brought out clearly with granule stains, especially with acid-fuchsin methylene green. With this stain the cytoplasm of most of the cells is found to be filled with eosinophilic granules, corresponding to the eosinophilic type of chromophil cell of the normal pars anterior of the hypophysis. The granules in cells undergoing degeneration do not stain.

A macroscopic and microscopic examination of the tumor by Dr. D. D. Lewis revealed no traces of the posterior lobe; serial sections, however, were not made.

#### DISCUSSION

The polyglandular syndrome is represented here by (1) a chromophil cell adenoma of the anterior lobe of the hypophysis, (2) a persistent and non-involuting thymus, (3) a colloid goiter with marked desquamation of the parenchymatous cells, (4) hypertrophy of the chromaffine cells of the adrenal medulla, (5) hyperplasia of the endothelial elements of the lymph-nodes and of the spleen, (6) enlargement of the pineal body, and (7) atrophy of the cells of Sertoli and of Leydig (assumed on the basis of the loss of sexual function, and from other similar case reports).



In this case the hypophyseal disturbance is the probable underlying cause of the anatomical and functional upsetting of the other internal secreting glands, and without doubt it is the cause of the acromegalic symptoms. In the present state of our knowledge concerning the real cause of the hypophyseal growth, the possibility of fundamental biochemical disturbance affecting all of the glands at once, is not to be denied. Noticeable pressure symptoms are often lacking in the early hyperplasia of the hypophysis, even though acromegalic changes have occurred; this is explained by the fact that a hyperplasia of the chromophil cells may occur without appreciable concomitant enlargement of the gland. Some cases of acromegaly terminate fatally soon after the appearance of the acral changes, while other cases continue over a period of twenty years without causing any pressure symptoms. In other words, chromophil cell hyperplasia may be very insidious in its onset, and without gland enlargement extended enough to cause focal symptoms, but at the same time causing gigantism or acromegaly. On the other hand, hyperplasia of the anterior lobe may come on suddenly with rapid growth of the chromophil struma, causing death before there are any symptoms manifest of disturbed gland function.

In the case reported here, although clinical symptoms did not appear until a year before the death of the patient, hypertrophy of the anterior lobe, at least of the chromophil cells, would seem to have been present for ten years or more, because the thymus shows no signs of even a beginning involution; normally the thymus undergoes involution at about the thirteenth year.

The hyperplasia of the lymphoid structures of the intestines and lymph-nodes as well as of the spleen, is indicative of Paultow's status thymo-lymphaticus. Cushing cites two such cases; Claude and Boudouin<sup>17</sup> cite another case. It is not probable that the lymphatic enlargement here is part of a general tissue hyperplasia, since the increase of the lymphoid structures is out of proportion to the enlargement of the other tissues.

It is not known precisely what hypophyseal disturbance is responsible for a persistent thymus, and why in some cases it is present while in other apparently similar cases, it is absent. In dogs that live some time after hypophysectomy, the thymus usually remains persistent or enlarged. In some, but not all, of the cases of long standing hypopituitarism there is a persistent thymus; on the other hand, in several instances of hyperpituitarism of the anterior lobe, such as in Cushing's Case 38, in the case of Claude and Boudouin, and in this case, a persistent thymus is also present. By hyperpituitarism of the anterior lobe is understood in these

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17. Claude, H., and Boudouin, A.: Etude histologique des glandes a secretion interne dans un cas d'acromegalie, *Comp. rend Soc. de biol., Paris*, 1911, lxxi, 75.

cases, a hyperplasia of the chromophil cells. In Graves disease the thymus is persistent and enlarged, according to Matti,<sup>4</sup> and to Capelle,<sup>18</sup> in 74 and 79 per cent. of the cases respectively; this would show that a hyposecretion of the anterior lobe of the hypophysis may be associated with a persistent thymus, since, according to Benda and Lewis, there is a decrease in the number of chromophil cells of the anterior lobe in cases of exophthalmic goiter.

Considerable variance of opinion exists concerning the relationship of the thyroid to the hypophysis. A condition of excess of colloid and of abundant parenchymatous desquamation, as in this case, has been found in all of Cushing's fifteen cases of dyspituitarism, where the thyroid was examined. It is his opinion that the thyroid is not functionally interchangeable with the hypophysis, but that both are simultaneously stimulated to hyperplasia by the same underlying biochemical disturbance.

In the case of giant Wilkins,<sup>19</sup> the thyroid gland weighed 112 grams and was of a colloid nature. In a chemical analysis of this thyroid Dr. H. G. Wells found that the total amount of iodine was 62.9 mg., or 2.28 mg. for each gram of the dried gland. In comparison to the iodine content of simple goiters from people living about Chicago and also from people living in the eastern states, Wells found that there is no difference in so far as the iodine content per gram weight of the dried gland is concerned. In simple goiters he found 1.5 to 2.5 mg. of iodine per gram of the dried gland.

Simpson and Hunter,<sup>20</sup> using the iodine content of the thyroid gland as the index to its activity, find no compensatory assumption of iodine by the hypophysis of sheep after thyroidectomy. Schonemann<sup>21</sup> examined the hypophyses and thyroids from 110 autopsies, including eighty-three cases of goiter. The results which are indicative of some relation between the glands are as follows:

27 cases of normal thyroid	.....	Hypophyses all normal.
30 cases of colloid thyroid	.....	Increase of the chromophil cells.
1 case of normal thyroid	.....	Increase of the chromophil cells.
33 cases of colloid thyroid	.....	Increase of the connective tissue.
10 cases of colloid thyroid	.....	Increase of the vascularity.
9 cases of colloid thyroid	.....	Increase of the colloid content.

Caselli<sup>11</sup> and Cushing both noticed hyperplasia of the thyroid gland after hypophysectomies; later, in the chronic stage, the thyroid became colloidal.

18. Capelle: Cited by Charles A. Parker. Note 3.

19. Bassoe, P.: Gigantism and Leontiasis Ossea, Trans. Chicago Path. Soc., 1901-1903, v, 231.

20. Simpson and Hunter: Does the Pituitary Body Compensate for Thyroid Insufficiency, Proc. Soc. Exper. Biol. and Med., 1910, viii, 5.

21. Schonemann, von A.: Hypophysis und Thyroidea, Virchows Arch. f. path. Anat., 1892, cxxix, 310.

In one case reported in the literature by Claude and Boudouin, the parathyroid glands were described as being four times as large as normal; no case of gigantism or of acromegaly could be found recorded in the literature in which parathyroid insufficiency, producing tetany, was evident.

Cushing has pointed out that in several of his cases, symptoms of hypoadrenalism were present, i. e., low blood-pressure, pigmentation of the skin, and hypoglycemia. In two other cases there were symptoms of hyperadrenalism, manifested by a high blood-pressure. In one of these latter cases, the adrenal medulla was the seat of a hyperplasia of the chromaffin cells; both adrenals together weighed 15.7 grams. In many other cases reported in the literature, hyperplasia of the medulla and vacuolization of the zona fasciculata are conspicuous.

As exemplified by this case, the interrelation of the hypophysis to the testicles and ovaries is a very close one. In hypophysectomized puppies, the testicles remain small and undeveloped, while in old dogs, hypophysectomy leads to impotence and atrophy of the testicles.<sup>22, 1</sup> Practically no secondary testicular change has followed experimental hyperpituitarism. Physiological hypertrophy of the hypophysis occurs during puberty, pregnancy and the climateric, indicating a close relation between the hypophysis and the sex organs.

Clinically, hypopituitarism, almost constantly associated with hypophysical tumors, leads to undeveloped ovaries and testicles, and to infantile sex characteristics, when occurring in young people; in adults, anaphrodisia, impotence, amenorrhea, and reversible sex characteristics result. It is probable that hyperpituitarism, such as occurs in early chromophil cell hyperplasias primary to acromegaly, leads to a transient sexual excitability. In Case 1 of Cushing's series, the history reveals an early excessive libido, while later there was impotence, after pressure symptoms and acromegaly had intervened. In my case, abnormal libido was the first sexual symptom noticeable.

The pineal gland in this case is of special interest, since it is over twice as large as normal. I have been unable to find any reference to its simple enlargement in connection with hypophyseal or with polyglandular disturbance. The physiological action of the pineal gland, if at all of importance, must be so during the latter part of intrauterine life and after birth, until the tenth or fifteenth year. At birth in man, it is the size of a grain of wheat; at the age of one year it is 3 mm. long, while at puberty it averages 7x5x4 mm., the largest size of all periods. From puberty on it becomes smaller, fibrous, cystic and filled with psammomata. Ontogenetically considered, it is the remains of the third eye or pair of

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22. Aschner, B.: Demonstration von Hunden nach extirpation der Hypophyse, Wien. klin. Wehnschr., 1909, xxii, 1730.

eyes, found now in one species of Australian lizard, the *Hatteria punctata*.<sup>23</sup> In Jordan's<sup>24</sup> excellent work on the development of the pineal body in sheep, where it is much larger than in other common mammals, he finds that in the 1.5-months-old embryo, there develop in the gland unbranched, blind alveoli, 80 microns long. These alveoli are composed of low columnar cells with an indistinct cytoplasmic outline; melanin granules are almost constantly found in the cytoplasm of these cells. At one half term (2.5 months), tufts of capillaries running in the connective tissue septa are invaginated into these tubules, forming structures not unlike the Malpighian bodies of the kidney. At term (5 months), the gland reaches its greatest development, being then 8x5x5 mm. in size, and containing numerous alveoli with many glomerular invaginations. Jordan could find no evidence of secretory activity other than the melanin granules. At 8 months after birth, the time of mating in sheep, the pineal body is still as large as at birth. After the first year it decreases in size, finally becoming small, sclerotic and cystic in old sheep. He concludes that its sphere of development up until puberty, together with its gland-like structure, makes it appear very much like a gland of internal secretion; on the other hand, all these phenomena of development can be interpreted on a phylogenetic basis.

Krabbe,<sup>25</sup> in the study of 100 human pineal glands, found what he interpreted as being secretory granules of a basophilic nature. The granules appear in the nuclei and are shed into the surrounding cytoplasm; this process occurs constantly up until advanced age. Kidd<sup>26</sup> believes that the pineal gland ceases in activity as the hypophysis waxes in function, having reference here perhaps to the fact that the chromophil cells of the hypophysis increase from birth on until 40.

Exner and Boese<sup>27</sup> extirpated the pineal gland in ninety-five young dogs, and observed several until puberty without noticing any physiological changes in their development. The injection experiments of von Cyon,<sup>28</sup> Howell,<sup>29</sup> Dixon and Halliburton<sup>30</sup> and others, with extracts

23. Bailey and Jelliffe: Tumors of the Pineal Body, THE ARCHIVES INT. MED., 1911, viii, 851.

24. Jordan, H. E.: The Histogenesis of the Pineal Body in Sheep, Am. Jour. Anat., 1911, xii, 249.

25. Krabbe: Sur le gland pineal chez l'homme, Nouv. iconog. de la Saltpétriére, 1911, p. 257.

26. Kidd, L. J.: Pineal Experimentation, Brit. Med. Jour., 1910, ii, 2202.

27. Exner, A., and Boese, J.: Ueber experimentalle Extirpation der Glandula-pinealis, Deutsch. Ztschr. f. Chir., 1910, cvii, 182.

28. Von Cyon, V.: Zur Physiologie der Hypophyse, Arch. f. d. ges. Physico., 1901, lxxxvii, 389.

29. Howell, C. M. H.: Tumors of the Pineal Body, Royal Soc. Med., London, March, 1910.

30. Dixon, W. E., and Halliburton, W. D.: The Pineal Body, Quart. Jour. of Exper. Physiol., 1909, ii, 233.



of the pineal body, were all practically negative. Dixon and Halliburton used extracts of adult sheep glands; as all evidence points to the fact that the function of the pineal gland is one of early life, it would be expected that extracts of adult pineal glands were inert.

Jordan and Eyster,<sup>31</sup> using fresh extracts of sheep pineal glands and injecting these into cats, dogs and sheep, obtained a slight fall of blood-pressure, greater than with extracts from other parts of the brain. Perfusion of two cats' hearts, using one gland to a liter of salt solution, produced a slight strengthening and quickening of the beat. In most dogs a short period of diuresis was produced by injection of acidulated extract. The author does not state whether or not he used glands from lambs or from sheep.

Foa<sup>32</sup> found in three young roosters from which the gland had been removed before the fifth week, that the testes and combs, at the eleventh month, were larger than those of the controls. These depinealized roosters crowed thirty-six days earlier, mated forty-seven days sooner, and were a little heavier than the controls. There were no macroscopic changes in the other glands. Biach and Hülles<sup>33</sup> removed the reproductive organs from kittens; seven months later they found that there was atrophy not only of the whole pineal gland, but also of its individual parenchymatous cells, as compared to the glands of normal kittens from the same litter.

Bailey and Jelliffe collected from the literature up until 1912, fifty-nine cases of pineal tumor, including adenomas, sarcomas, adenosarcomas, teratomas, glomas, endotheliomas and carcinomas. Four of these fifty-nine cases, namely, those of Ogle,<sup>34</sup> a sarcoma; Oestreich and Slawyk,<sup>35</sup> a psammomata cysticus; Marburg,<sup>36</sup> a teratoma, and Frankel-Hochwart,<sup>37</sup> a teratoma, all under 10 years of age, had developed the remarkable syndrome of precocious sexual maturity. For example, in Frankel-Hochwart's case, the boy at the age of 3 began to grow rapidly, so that at 5 he had reached the size of a boy of 7 years; his mind dwelt on adult subjects; his penis was large, while pubic and axillary hair were present; erections

31. Jordan, H. E., and Eyster, J. H. E.: *The Physiological Action of Extracts of the Pineal Body*, *Am. Jour. Physiol.*, 1911, xxix, 115.

32. Foa, C.: *Hypertrophy of Cocks' Combs and Testes Following Removal of the Pineal Gland*, *Arch. ital. de biol.*, Turin, 1912-1913, lvii, 233.

33. Biach, P., and Hülles, E.: *Ueber die Beziehungen der Zirbeldrüse zum Genitale*, *Wien. klin. Wchnschr.*, 1912, xxv, 373.

34. Olge, C.: *Sarcoma of the Pineal Body*, *Trans. Path. Soc. London*, 1899, 1.

35. Oestreich and Slawyk: *Risenwuchs und Zirbeldrüsen geschwulst*, *Virchows Arch. f. path. Anat.*, 1899, clvii, 475.

36. Marburg, O.: *Zur Kenntnis der normalen und pathologischen Histologie der Zirbeldrüse; Die Adipositas Cerebralis*, *Deutsch. Ztschr. f. Nervenh.*, 1909, xxxiv, 114.

37. Frankel-Hochwart, von L.: *Ueber Diagnose der Zirbeldrüsenumoren*, *Deutsch. Ztschr. f. Nervenh.*, 1909, xxxvii, 455.

were frequent; his voice had changed. At 9 years he was as large as a boy of 15, except that his genitals were those of a boy of 17 or 18.

A case reported by Cushing of a boy 8 years old with similar symptoms of precocious sexual development, may be included in this class of pineal cases, although exploratory operation revealed nothing obviously abnormal with the pineal body.

Marburg was the first to lay emphasis on this syndrome. The metabolic symptoms, according to him, are (1) precocious sexual development due to hypopinealism, (2) universal adiposity due to hyperpinealism, and (3) cachexia due to apinealism. Adiposity has been found in about ten cases of pineal tumor unassociated with sexual disturbance; this adiposity may be very well explained, aside from whatever specific effect the pineal gland may have in governing carbohydrate metabolism, by a hyposecretion of the posterior lobe of the hypophysis, resulting in turn from a third ventricle hydrocephalus; occlusion of the duct of Sylvius by tumors located in the pineal region frequently results in hydrocephalus of the third ventricle, a condition often associated with adiposity.

Whether the enlargement of the pineal body, in the case reported here, is due to some primary biochemical disturbance, or is secondary to the hypophyseal disturbance, cannot be determined. If the pineal gland is one of the glands of internal secretion, and in all probability it is during early life, its abnormal enlargement here, as but one manifestation of a polyglandular syndrome, is of considerable significance.

I wish to acknowledge many thanks to Dr. H. Gideon Wells, and to Dr. Dean D. Lewis for their valuable suggestions in the preparation of this paper.

## THE FUNCTIONS OF THE DIAPHRAGM AND THEIR DIAGNOSTIC SIGNIFICANCE \*

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CLEVELAND

A clear understanding of the respiratory rôle of the diaphragm is necessary to analyze the respiratory movements in the thorax and abdomen. In former years the inspiratory excursion of the diaphragm was grossly exaggerated by estimating the excursion of the lower borders of the lungs as a measure of phrenic movement. These were misleading because the descent of the lung in the pleural sinus to an extent of 6 centimeters may be accomplished by the diaphragm descending or withdrawing from the thoracic wall only a distance of 1 centimeter.

The inspiratory descent of the anterior hepatic border is an exaggeration of phrenic movement because the liver rotates on a transverse axis during inspiration and is not depressed in toto during inspiration. This axis of rotation is marked by the posterior hepatic border or that liver portion which is not covered by peritoneum. This part of the liver is fixed by reflections of the peritoneum from the liver to the under surface of the diaphragm and posterior abdominal wall from the superior and inferior surfaces, respectively, of the liver.

Inspiratory movements of the diaphragm, therefore, rotate the liver forward and downward. The rotating force is applied on the posterior and superior portion of the upper surface of the liver and therefore the excursion of the anterior border of the liver corresponds to the excursion of a lever of the third class, the fulcrum being at the posterior hepatic border and the power applied at the posterior portion of the upper surface of the liver. So the descent of the lower pulmonary and anterior hepatic borders are gross exaggerations of phrenic excursion.

In recent times the fluoroscope has given us more accurate conceptions of the inspiratory descent of the diaphragm. A very good example of this magnification of phrenic descent is seen in pericarditis with effusion. Several years ago a patient with purulent pericarditis of pneumococcus origin came under my observation. The liver in this instance presented a great rounded tumor on the right side of the abdomen with its anterior border rotated downward in the iliac fossa. Paracentesis of the pericardial sack was performed, and although only 700 c.c. of pus were

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\* Submitted for publication May 17, 1913.

\* Read before the meeting of the Association of American Physicians, Washington, D. C., May, 1913.

removed, when the aspiration was finished, the anterior border or edge of the liver occupied its normal position at the right costal border.

All discussions of the function of the diaphragm which have appeared in recent years contain recognition of two movements at the base of the thorax which are caused by diaphragmatic contractions: (1) retraction of the hypochondria, and (2) widening of the subcostal angle or divergence of the costal borders.

Keith,<sup>1</sup> in his article on "Respiration in Man," refers to Duchenne's experiments and deductions which amounted to proving that diaphragmatic action contributed to widening of the subcostal angle and spreading of the hypochondria by virtue of the piston action of the liver, spleen and stomach, which are driven downward and forward, thus spreading the upper lateral abdominal boundaries. All modern works in physiology and anatomy refer to Duchenne's experiments and unite in maintaining the validity of his deductions.

When one attempts routinely to analyze the respiratory excursion of the thoracic walls in a service of patients with varied thoracic and abdominal conformations and with varied thoracic and abdominal diseases, it will be found that Duchenne's explanation is not tenable.

1. It is proposed to show in the following discussion that the resultant of phrenic contraction always tends to narrowing of the subcostal angle, retraction of the lower end of the sternum and retraction of the internal portions of the hypochondria above the level of the ninth costal cartilages.

2. Although the action of the diaphragm is correlated with the action of the scaleni and intercostal muscles, the diaphragm is the antagonist of the other muscles in their efforts to enlarge the transverse and antero-posterior diameters of the base of the thorax, and also acts as an antagonist to the tendency of these muscles in their inspiratory activity to lessen the longitudinal diameter of the thorax and lengthen the longitudinal diameter of the abdomen.

3. There is a two-fold purpose in the contraction of the diaphragm, viz., to maintain the integrity of the longitudinal diameter of the thorax against the inspiratory effect of the scaleni and intercostals, and also to enlarge the longitudinal diameter of the thorax.

4. The resulting variations in respiratory movements of the lower end of the sternum, subcostal angle and costal borders from the ninth rib to the subcostal angle, the epigastrium and hypochondria, all depend on the higher or lower positions of the diaphragm.

5. When the diaphragm is pushed upward it is placed at a mechanical disadvantage in approximating the points of origin and insertion of its muscle, viz., the central tendon, sternum and costal borders.

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1. Keith: *Further Advances in Physiology*, 1909, p. 182.



But when the diaphragm is displaced downward the muscle is placed at a mechanical advantage for its effect on the movement of these structures.

6. However high or low the diaphragm may be placed, so long as its muscle is active the longitudinal diameter of the thorax is either maintained or enlarged by its action.

Although all these propositions seem very simple and are easily explained, the want of a satisfactory understanding of the function of the diaphragm has made it impossible to make a critical analysis of respiratory movements of the thorax in any and all diseases.

The so-called Litten diaphragm phenomenon is of service in estimating the excursion of the lower border of the lungs, but it is such a small part of the many evidences of respiratory movement at the lower thoracic aperture, that when it is absent the phenomenon is not of any diagnostic service. Several monographs<sup>2</sup> on the diaphragmatic function have been published from German sources, but they deal only with the effect of phrenic contraction on the pleural sinus and treat the excursion of the costal angle evasively. The more important manifestations of diaphragmatic activity are disregarded because the antagonistic relations between the diaphragm and scaleni have not been taken into account.

In the dog in health and disease the diaphragm and its antagonists produce respiratory movements of the lower end of the sternum, subcostal angle, costal borders, epigastrium and hypochondria, identical with those seen in human beings. In man, spasm of the diaphragm causes retraction of the lower end of the sternum, narrowing of the subcostal angle and violent approximation of the hypochondria toward the median line. When this function is seen in diaphragmatic pleurisy or mediastinitis in man we are in doubt about the movements resulting from diaphragmatic action alone. The transverse abdominal muscles may also contribute to the movements. If the linea alba of the dog is slit from the sternum to the pubes and the abdominal walls on both sides are cut transversely to the quadratus lumborum then muscular activity of the abdominal walls can be eliminated as a factor in movements of the costal borders.

After these preparatory incisions, electrodes are placed on the right and left leaflets of the diaphragm. When the diaphragm is then stimulated by a strong electric current the sternum, subcostal angle, costal borders and hypochondria exhibit the same movements as are seen during phrenic spasm in man. When the three roots of the phrenic nerve of the dog are cut on both sides so the diaphragm is completely paralyzed, then the inspiratory widening of the subcostal angle and flaring of the hypo-

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2. Zabel, Erich: *Das Spiel des Zwerchfells*, Berlin, 1906, p. 176; Eppinger: *Allgemeine und Spezielle Pathologie des Zwerchfells*, Wien, 1911, p. 252.

chondria during inspiration are much exaggerated over the same movements which occur when the phrenic nerves are intact.

A few months ago we had a child at Lakeside Hospital whose arms and legs and diaphragm were completely paralyzed from poliomyelitis anterior. The scaleni and intercostal muscles were intact. The respiratory movements were identical with those of the dog whose phrenic nerves were severed.

The subcostal angle showed an exaggerated widening during inspiration. The costal borders and hypochondria diverged from the median line, and the epigastrium was retracted during inspiration. In other words, the scaleni and intercostals had no antagonist to modify their inspiratory movements of the hypochondria and costal borders.

A man, 47 years old, was operated at Lakeside Hospital six months before his second admission to the hospital on account of empyema of the gall-bladder; drainage was established and in about two months the drainage from the gall-bladder ceased, the sinus closed and the patient was perfectly comfortable during a period of four months, when suddenly he was seized with chills and fever and severe pain in the right hypochondrium and over the right deltoid muscle. A week later he was admitted to the medical wards and presented all the characteristic physical signs of a large amount of fluid in the right pleural cavity. The right thorax was larger than the left. The right side was flat on percussion from the clavicle to the edge of the liver which was located 2 inches below the costal border in the nipple line. The physical signs of auscultation and percussion were characteristic of a pleural cavity filled with fluid. The one sign which was at variance with thoracic empyema was the fact that the affected side had a larger excursion, and particularly the right costal border diverged much more from the median line than did the left costal border. This fact indicated a loss of function of the diaphragm and suggested that an infraphrenic abscess had ruptured through the diaphragm into the right pleural cavity, or at least a myositis of the diaphragm had resulted in a loss of its muscular function. For, had the diaphragm been displaced downward by the empyema and had the phrenic musculature been left intact then the activation of the phrenic muscle would have fixed the right costal border so as to prevent its divergence from the median line; or the right costal border would have been drawn toward the median line during inspiration. Evidently the scaleni and intercostal muscles of the right side were not opposed by the normal antagonistic action of the diaphragm. Unfortunately, the patient's critical condition at the time of operation did not admit of definitely determining whether there was or was not an opening through the diaphragm. Two weeks after the empyema was drained the patient's costal borders moved symmetrically during inspiration.

Another patient developed signs suggesting thoracic empyema following appendicitis. The base of the right thorax was dull, tactile fremitus was diminished. Auscultation revealed a diminution of the intensity of the respiratory sounds attended with elevation in pitch of the inspiratory and expiratory sounds.

All these physical signs were strongly suggestive of thoracic empyema, but one sign alone gave the key to the diagnosis. The costal border of the affected side had a greater lateral excursion than the sound side. This fact could have only one interpretation, and that was a loss of the normal antagonism of the diaphragm to the action of the scaleni and intercostals of the affected side.

In conjunction with the other physical signs mentioned, this sign indicated an upward displacement of the diaphragm and led to the diagnosis of a subphrenic abscess.

An operation was performed to establish drainage and the entire accumulation of pus was found beneath the diaphragm.<sup>3</sup>

Upward displacement of the diaphragm diminishes the inspiratory restraint of the costal borders because the more its muscle is curved the less will be the resulting traction on its sterno-costal attachments. Conversely, the flatter the diaphragm the greater will be the resulting traction on its sterno-costal attachments.

When hepatic enlargements displace the diaphragm in an upward direction, then the right costal border will exceed the left costal border in its inspiratory divergence from the median line. Patients with acute jaundice who have swollen livers show this disparity in movement. After the jaundice has subsided and the diaphragm resumes the original position then the two costal borders are seen to have a symmetrical excursion.

When the liver is enlarged in an upward direction sufficiently to displace the lower border of the lung a distance of one intercostal space this increase in the excursion of the costal border is distinctly visible. Paresis of the diaphragm or accentuation of the convexity of the diaphragm will serve to increase the inspiratory divergence of the costal border from the median line. An upward displacement of the diaphragm will not serve to increase the movement of the costal border on the affected side if the displacement is caused by an obsolete pleurisy with synechia between the diaphragm and the thoracic wall. This is particularly true when the site of the synechia is in the anterior portion of the pleural sinus.

A patient who recovered from bilateral pleurisy had the usual characteristic signs of obsolete pleurisy, viz., dullness, diminished excursion,

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3. This observation was made by Dr. H. L. Taylor, resident physician at Lakeside Hospital.

diminished tactile fremitus and lessened intensity of the respiratory murmur without elevation in pitch of the respiratory sounds or any adventitious sounds. The right costal margin moved away from the median line during inspiration, but the left costal border moved toward the median line during inspiration. The physician who attended the patient during his illness said the most persistent and most intense dulness and resistance was over the left inframammary region where the most marked signs of thickened pleura and synechia persisted.

This evidence of increased diaphragmatic retraction of the costal border suggested that the diaphragm had acquired a mechanical advantage in its traction on the costal border because there was fixation of the diaphragm to the thoracic wall above the costal border, thus giving approximately a straight line to the phrenic muscular strands from the central tendon to the left sterno-costal region.

This view was confirmed by the following very simple experiments:

The abdomen of a dog was opened, and to avoid the possibility of admitting air to the pleural cavity a thin strip of wood was placed against the under right side of the diaphragm, so that the diaphragm over the site of the pleural sinus from the right sternal border to the anterior axillary line was brought into contact with the thoracic wall by a clamp which was applied to the outer side of the thorax and the inner surface of the strip of wood. This gave a high insertion of the diaphragm and resulted in fixation and retraction of the right costal border during inspiration. The same effect on the inspiratory excursion of the costal border was procured by fixing the diaphragm to the ribs about 2 inches above the costal margin by means of silk ligatures which were passed through the diaphragm and thoracic wall with very thin needles. When the ligatures were released the costal border resumed its original inspiratory divergence from the median line.

This manner of upward displacement of the diaphragm converts the line of traction from a curved to a straight line, thus placing the diaphragm at a mechanical advantage, whereas an upward displacement of the diaphragm which accentuates the curve of the line of traction (as occurs in hepatic enlargement) will place the diaphragm at a mechanical disadvantage. The movements of the costal borders and lower end of the sternum are much more readily affected of course by varying elevations and depressions of the anterolateral strands of the diaphragm than the changes in the elevation of the posterior strands of the diaphragm. This is apparent from clinical experiences and also from trials made in the experiment on the dog which I have just described. When the posterior portion of the diaphragm was fixed to the thoracic wall over the site of the pleural sinus, the inspiratory widening of the subcostal angle was not modified.



Thus far the discussion has dealt with clinical and experimental evidences which show the subcostal angle is enlarged during inspiration by the action of the scaleni and intercostal muscles, and also that impairment of the diaphragmatic muscle or paresis from disease or cutting the phrenic nerves or accentuation of the arch of the diaphragm will contribute to the inspiratory widening of the subcostal angle. We can now see the errors involved in the interpretation of Duchenne's experiment of driving the diaphragm upward by bandaging the abdomen of the dog and thus increasing the longitudinal diameter of the abdomen at the expense of the longitudinal diameter of the thorax. The accepted interpretation of this experiment is, the diaphragm thus being prevented from encroaching on the longitudinal diameter of the abdomen by its contraction, expends its force in elevating the costal borders and widening the subcostal angle.

The correct interpretation of Duchenne's experiment lies in the fact that an increase of the convexity of the diaphragm without altering its insertion in the antero-lateral thoracic wall, places the diaphragm at a mechanical disadvantage and thus contributes to widening of the subcostal angle by the action of the scaleni and the intercostal muscles.

Downward displacement of the diaphragm will place the diaphragm at a mechanical advantage in restraining the divergence of the costal borders, and thus causes a diminution of the inspiratory widening of the subcostal angle, and may cause retraction of the lower end of the sternum, narrowing of the subcostal angle and approximation of the hypochondria toward the median line.

My attention was first called to these phenomena in pulmonary emphysema. A moderate degree of pulmonary emphysema will cause the pleural sinuses to be filled with the enlarged pulmonary borders and still the emphysema will not be severe enough to cause cyanosis or an increase of the  $\text{CO}_2$  content of the alveolar air above 5.3 per cent. Under these conditions there will still be a distinct widening of the subcostal angle during inspiration. If the degree of emphysema increases of course the lower borders of the lung can descend no further than the bottom of the pleural sinus, but farther flattening of the diaphragm causes the subcostal angle to be narrowed during inspiration and the anterior hypochondria will be approximated toward the median line during inspiration. When emphysema is sufficiently severe to cause cyanosis, so that the alveolar air will contain 6.5 per cent. or more of  $\text{CO}_2$ , then the subcostal angle will not be enlarged or it will be diminished during inspiration.

Variations of degree in pulmonary emphysema offer very good opportunities to see just when the critical point in flattening of the diaphragm is obtained.

The first step in emphysema reveals the pleural sinus filled with lung; then as the flattening of the diaphragm progresses, the critical point is attained when the subcostal angle remains constant during inspiration, and further descent of the diaphragm is attended with inspiratory narrowing of the subcostal angle and inspiratory approximation of the hypochondria. The last condition is always seen when the alveolar air obtains a percentage of CO<sub>2</sub> as high as 7.5 per cent.

When pulmonary emphysema is attended with cyanosis and these changes in the respiratory movement of the subcostal angle and hypochondria are not apparent, then it can be said with certainty that the cyanosis is not due to impairment of lung ventilation from emphysema alone, but the cyanosis must then have a contributory cause in some other factor which impairs either internal respiration or the respiratory function of the lung, which is quite distinct from the ventilatory function. Conversely, when an emphysematous patient has marked inspiratory narrowing of the subcostal angle and cyanosis is absent, then some other factor besides enlargement of the lung must contribute to the flattening of the diaphragm. Enlargement of the pericardial sac causes inspiratory narrowing of the subcostal angle.

A small pericardial effusion may be demonstrated by other physical signs when the phrenic displacement is so slight that it allows inspiratory widening of the subcostal angle. As the effusion increases the critical point is reached when the subcostal angle remains constant during all respiratory phases and the epigastrium is protruded during inspiration. When accumulation of fluid in the pericardial sac progresses there will be inspiratory narrowing of the subcostal angle and inspiratory retraction of the epigastrium.

It is this stage of diaphragmatic depression which has often been interpreted as phrenic palsy in pericarditis. The diaphragm is not paralyzed, but it is activated during inspiration, and because of its downward displacement retracts the hypochondria and narrows the subcostal angle. Owing to the flattening of the diaphragm, its activation does not cause a further lengthening of the thorax, but the longitudinal diameter of the thorax is maintained and the epigastrium is retracted because the thoracic cage in toto is elevated. This elevation of the thoracic cage enlarges the capacity of the hypochondria; consequently the epigastrium is retracted.

Within a day's time the pericardial effusion may be absorbed sufficiently to cause a return to the normal type of respiratory movement of the subcostal angle and the epigastrium. This lack of respiratory excursion of the diaphragm attended with inspiratory retraction of the epigastrium has been incorrectly described as paralysis of the diaphragm in pericarditis. There can be no paralysis of the diaphragm when the

subcostal angle is narrowed during inspiration. Paralysis of the diaphragm contributes to the inspiratory widening of the subcostal angle. We have had ample opportunity to confirm at Lakeside Hospital this effect of pericardial effusion and the inspiratory movement of the subcostal angle and costal borders. A patient with pericarditis who was frequently tapped always exhibited the phenomena above described before and after each paracentesis.

These phenomena are specifically described because one finds in all neurological works very unsatisfactory discussions of the symptoms of paralysis of the diaphragm. And where specific statements are made, as in *La Pratique Neurologique* by Guillaing,<sup>4</sup> we find the symptoms of phrenic paralysis are described as showing "inspiratory retraction of the hypochondria and expiratory expansion of the hypochondria which is the opposite of what is normally seen." This error has evidently arisen from mistaking the evidences of a flattened diaphragm for evidences of diaphragmatic palsy.

Cardiac enlargement may alter the respiratory excursion of either the left or both costal margins. If the enlargement is to the left of the median line, only the left margin is affected; if the enlargement is to the median line, as occurs in mitral stenosis, then the subcostal angle will be symmetrically narrowed during inspiration.

Thus far only those conditions which cause a widespread flattening of the diaphragm (as in emphysema), or those which cause purely a local flattening of the sterno-costal fibers of the diaphragm have been described.

Filling of the pleural sinus laterally and posteriorly will also modify the inspiratory excursion of the costal border on the affected side. This effect is of great service in recognizing pleurisy with effusion and pneumothorax, as both conditions will result in fixation of the costal border or an inspiratory traction of the costal border toward the median line.

For example, a young girl 18 years old, had a pleurisy with effusion on the right side which was unattended with pain. The fluid occupied only the posterior aspect of the pleural cavity. During inspiration the left costal border diverged from the median line. The right costal border was drawn toward the median line during inspiration. Two hundred and seventy c.c. of fluid was aspirated and then the right costal border diverged from the median line.

Pleuritic pain accompanying an inspiratory movement will inhibit the excursion of the costal margin during inspiration, but this is quite different from restraint of the costal border in pleurisy with effusion. When pleuritic pain is a factor, the scaleni and intercostal muscles of the affected side are inhibited in their action, so of course the costal

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4. Marie, P.: 1911, p. 650.

border will move very little, but the costal border is not drawn toward the median line. The important point to bear in mind is to determine the movement of the costal border when the actions of the scaleni and intercostals are not inhibited.

This movement of the costal border is frequently of great service in differentiating between pulmonary infiltration of the lower lobe of a lung and pleurisy with effusion, and also in differentiating between an obsolete pleurisy and pleurisy with effusion when the obsolete pleurisy does not involve the sterno-costal portion of the diaphragm.

Consolidation of the lower lobe of the lung and obsolete pleurisy may diminish the excursion of the costal margin, but there will be some movement and that will be away from the median line, and not toward it, as occurs in pleurisy with effusion.

Pneumothorax causes inspiratory traction of the costal border toward the median line on the affected side, as can be readily seen in man and produced in dogs by the admission of air to a pleural cavity. Air was admitted to the right pleural cavity of a dog and directly the costal margin of the right side was seen to move toward the median line during inspiration.

All the roots of the right phrenic nerve were then cut and directly the right costal border diverged from the median line during inspiration, although the diaphragm occupied a very low position. The diaphragmatic attachments to the right costal border were then cut and the lateral excursion of the right costal border during inspiration was greatly increased.

Keith<sup>1</sup> says, "The writer, from observations made by the x-rays, has come to regard a free movement of the subcostal angle as an index of a free action of the diaphragm." This statement is literally true, but not in the sense in which Keith interprets the phenomenon. If the diaphragm is activated in a normally arched position or in a position higher than normal, there will be a marked excursion of the diaphragm and inspiratory widening of the subcostal angle. In this sense activation and excursion of the diaphragm are attended with widening of the subcostal angle, but the inspiratory widening of subcostal angle is not due to activation of the diaphragm, but to activation of the muscles which lift the thoracic cage. When the convexity of the diaphragm is flattened its activation will be quite as strong as when the diaphragm has a maximum convexity, but phrenic activation in a lowered position manifests itself in fixation or narrowing of the subcostal angle.

Briefly stated, movements of the costal borders during respiration give valuable aid in differentiating between supraphrenic and subphrenic disease. Also in differentiating between pulmonary consolidation and



pleurisy with effusion, and in differentiating between obsolete pleurisy and pleuritic effusion.

Costal border movements enable us to form a comparative estimate of a degree of pulmonary emphysema which exceeds mere filling of the pleural sinus with aerated lung. Movements of the costal borders give indication of the amount of fluid in a pericardial sack and also contribute to an appreciation of cardiac enlargement and an appreciation of the relative enlargements of the two ventricles. Unlike the diaphragm phenomenon of the pleural sinus, these movements of the costal borders and the subcostal angle are always present and can be accurately observed whatever the disease may be and however thick may be the panniculus adiposus. So these signs have a much broader and more accurate diagnostic significance than the so-called Litten diaphragm phenomenon.

In conclusion a word of caution should be added. In making an estimate as to whether a costal border is stationary or diverges from, or is drawn toward the median line, the examiner should apply his thumbs symmetrically placed along the costal borders to serve as indicators. Inspection alone without the aid of palpation of the borders may lead to confusion between elevation of the thoracic cage and widening of the subcostal angle. The thoracic cage may be strongly elevated and still the subcostal angle remain the same size. In fat persons, asymmetrical movements of the costal borders may be obscured without the aid of indicatory palpation.

I wish to acknowledge much helpful assistance in animal experiments from Dr. H. L. Taylor, resident physician in Lakeside Hospital, and from Dr. R. G. Pearce of the department of physiology in Western Reserve Medical School.

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# ON THE DEMONSTRATION OF VARIATIONS IN THE THYROID COLLOID IN CONDITIONS OF HYPER- AND HYPOTHYROIDISM \*

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The experiments here reported were undertaken in an attempt to demonstrate functional variations of the thyroid. From an analogy with other glands of the body, such variations probably exist in different individuals, and also in the same individual under different conditions. Heretofore there has been no method sufficiently dependable to follow even the extremes of activity of the gland, not to speak of the many possible intermediate stages. Until a satisfactory method is developed, no definite knowledge is obtainable of the true character of the various conditions supposedly referable to abnormal states of the thyroid gland.

One of us<sup>1</sup> has for a considerable time been interested in the development of a biological test for the thyroid secretion. Attempts are being made to use the cretin rabbit for this purpose, yet by the technic so far employed satisfactory results have not been obtained.

The Hunt acetonitril test, though valuable for certain lines of attack, has failed in the hands of Carlson and Woelfel,<sup>2</sup> Hunt<sup>3</sup> and Lussky<sup>4</sup> to determine any constituent of the thyroid product in the blood of dogs, rabbits or guinea-pigs in any experimental condition of hyperthyroidism. With the blood of clinical cases of exophthalmic goiter, Hunt obtained positive results in two out of three cases. Carlson and Woelfel could not get a positive reaction in the case studied by them, nor in Carlson's own blood after having taken thyroid till disagreeable symptoms arose.

Very recently one of us<sup>5</sup> has shown Mallory's connective tissue stain, when applied to the colloid in tissues fixed in Zenker's solution, to be capable of indicating variations in the iodine content. It was thought that the iodine variations might well be parallel to, or dependent on, a functional thyroid activity, and hence by such a method, variations in thyroid activity might well be followed. The work reported in this paper is based on the estimation of relative amounts of iodine in thyroid colloid as

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\* Submitted for publication June 16, 1913.

<sup>1</sup> From the Departments of Pathology and Pharmacology, University of Wisconsin.

1. Tatum, A. L.: Jour. Exper. Med., 1913, xvii, No. 6.

2. Carlson, A. J., and Woelfel, A.: Am. Jour. Physiol., 1910, xxvi, 32.

3. Hunt, Reid: Jour. Am. Med. Assn., 1907, xlix, 240.

4. Lussky, H. O.: Am. Jour. Physiol. 1912, xxx, 63.

5. Jones, A. P.: Jour. Exper. Med., 1913, xvii, No. 5.

indicated by the reaction with the modified Mallory's stain. Although other molecular groups than those containing iodine may stain blue with the stain used, yet the rough parallelism of amount of blue staining colloid to the analyzed iodine content of the gland, together with increased blue colloid on iodine administration, indicate very strongly that the blue staining reaction is due to the iodine in the colloid. Obviously we are entirely in the dark as to the character of the iodine combination, either in the colloid of the gland or in the circulation; but even if subsequent work showed that this staining reaction is not due to iodine, the least that can be said is that the extent of this staining reaction runs parallel to the iodine concentration. We are compelled to follow the unknown molecule by the single element iodine, and that we determine only as it occurs in the thyroid colloid.

The manipulative procedures were the following: After aseptically removing a small piece of one thyroid lobe, rabbits were fed on commercial desiccated sheep thyroids<sup>6</sup> for three or four days until, with very moderate doses, distinct toxic signs were seen. Then another small piece was removed from the remaining intact lobe, after completion of the period of thyroid administration. The thyroid colloid showed in every case a marked increase in the blue staining reaction.

These thyroid-fed animals were bled, and serum was prepared by coagulation and centrifugalization. This serum was injected intravenously into a test rabbit from which a small piece of thyroid had previously been removed for control. Forty-eight hours later a portion of the remaining intact lobe was removed, fixed in Zenker's solution and stained by the method above mentioned.

This procedure results in an accumulation of iodine in the thyroid of the test animal, as indicated by the increased area of blue staining colloid. This result was constant in all but a single case (see table), in which the second portion of thyroid was removed after twenty-four instead of forty-eight hours. Several animals showed a positive reaction at twenty-four hours, but in view of the apparent exception, we thereafter removed the second portion only after forty-eight hours, with positive results in every case. The thyroid colloid of the intoxicated animal gives the same iodine reaction seen in the test animals which had received intravenously serum from the hyperthyroid animal. This demonstrates the presence of iodine in the blood of the hyperthyroid animals,<sup>7</sup> and since the colloid takes iodine from the blood, the latter may be considered hyperiodized with respect to the colloid itself. The thyroid-fed animals were

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6. The material used was very kindly supplied to us by Parke, Davis & Co., for which we wish to express our appreciation.

7. In this paper we shall use the term "hyperthyroid" reservedly to mean that condition produced directly or indirectly by the feeding of thyroid products.

undoubtedly hyperthyroid, as shown by the toxic symptoms. From these results we believe that the same qualitative conditions of thyroid excess may well exist in both animals, differing mainly in degree. While the thyroid-fed animals were seen to be definitely intoxicated, the animals receiving hyperthyroid serum showed no apparent change other than the difference in staining reaction of the colloid. Pure inorganic iodid medication does cause a positive thyroid colloid reaction as shown by Jones, but relatively enormous doses were given (1.5 to 4 gm. iodine as potassium iodid). In our feeding experiments the iodine administered as thyroid iodine was approximately 4 mg. The commercial thyroid extract used was guaranteed to contain not less than 0.2 per cent. iodine. We did not check this determination, but did find that there was no inorganic iodine present.

It was thought advisable to parallel the thyroid feeding experiments with a series in which there was given an amount of inorganic iodine equal to the amount of organic iodine administered in the form of thyroid extract. The results, as here tabulated, show that so small an amount of inorganic iodine gives practically no change:

Number	Treatment	Result
JT 47	7 mg. sodium iodid in six days . . . .	No change
JT 48	4 mg. sodium iodid in six days . . . .	Slight increase
JT 49	No treatment . . . . .	Slight increase
JT 50	4 mg. sodium iodid in six days . . . .	Decrease
JT 51	4 mg. sodium iodid in six days . . . .	Slight increase

Jones administered from 2 to 5 gm. potassium iodid and still found some yellow colloid. In the thyroid-fed animals all of the colloid gave a deep blue reaction. This shows that iodine accumulates in the colloid much more readily when in a thyroid combination than as inorganic iodid. Whether the accumulated iodine comes from the administered material directly, or from other tissues under the stimulus of this administered material, we have not determined. Certain it is, however, that both commercial thyroids and inorganic iodids produce a similar reaction in the thyroid when administered to the animal *per os*. From a study of the Jones data on an iodine basis, the commercial thyroid product is seen to be more active with respect to production of a blue staining colloid than one thousand times as much iodine when given as potassium iodid. On the other hand, Jones could find no blue staining colloid in certain glands which, on analysis by the Baumann-Oswald method, gave some, though small, amounts of iodine. The advantage of this method obviously is that it is a microchemical test and is applicable to minute sections of thyroid, the loss of which would produce only a negligible effect on the unremoved portion. It has the disadvantage of necessarily assuming the thyroid colloid to be alike throughout the various areas of the whole gland. This, together with the fact that there is, in all prob-



ability, a small amount of iodine in the cells of the thyroid, probably explains the discrepancies in Jones' data compared with the actual iodine content. The results in our present series are so constant as essentially to eliminate this factor.

Since there is no known method of standardization of desiccated thyroid preparations on any basis other than that of their iodine content, it is not known definitely whether hyperthyroidism can be produced equally well with an iodine free thyroid product. The balance of opinion, however, is in favor of the necessity of the iodized component.<sup>8</sup> Certain it is that alkali iodides administered in equivalent dosages produce none of the signs of intoxication seen in experimental hyperthyroidism.

Serum from thyroidectomized rabbits when injected into normal test animals should produce in the blood of the latter a relative deficiency of thyroid products, and following physical laws should cause an outflow from the gland colloid. On the other hand, such serum might contain a physiological stimulus in the form of certain metabolic products which act specifically on the thyroid gland. Whatever the mechanism, we should expect either a decrease in the iodine of the gland or no change, depending on the individual ability to recuperate from the increased demands for thyroid secretion. This is just what was found. In four instances there appeared no demonstrable change, but in six of the animals there was observed a marked decrease in the iodine stain.

A mixed serum from several normal rabbits might be expected to produce in a series of normal animals any of the three possible changes: increase, decrease, or no change of the iodine content, dependent on whether the thyroid activity of the individual test animal was above, equal to, or below the common level of activity determined by the donors. This was the result obtained.

The accompanying tables need but little explanation. All rabbits marked with a common letter have received similar treatment and belong to the same series. A mixed serum from several animals was used in order to reduce to a common level all individual variations.

It is seen that of ten rabbits receiving serum from hyperthyroid rabbits, all show an increase of iodine except one, which was tested twenty-four hours after injection. This exception indicates that twenty-four hours is rather too short a time for demonstrable iodine changes to take place, though several gave positive tests within that time. At forty-eight hours there were no exceptions. How long the excess may be retained we have made no attempt to determine.

None of the rabbits receiving hypothyroid rabbit serum showed an increase in iodine, though in four instances there was no change, while the greater number showed an actual decrease.

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8. Stoland, O. O.: *Am. Jour. Physiol.*, 1912, xxx, 37.

Of fifteen animals receiving normal rabbit serum, four responded with an increase in colloid iodine; the rest have shown either no change or a decrease.

All rabbits receiving the commercial desiccated thyroid *per os* showed increased iodine.

The rôle of the iodine in the thyroid gland is no more understood than that of the thyroid itself. The iodine may play a rôle in the thyroid secretion roughly comparable to the iron of hemoglobin. Hemoglobin is a non-toxic compound. Hematin, on the other hand, is quite toxic.<sup>9</sup>

TABLE SHOWING IODINE RESPONSE IN RABBITS RECEIVING VARIOUS SERUMS

Rabbits Receiving 5 c.c. Normal Rabbit Serum.				Rabbits Receiving 5 c.c. Hyperthyroid Rabbit Serum.				Rabbits Receiving 5 c.c. Thyroidectomized Rabbit Serum.			
No.	Wt. Gms.	Time, Hours.	Iodine Response	No.	Wt. Gms.	Time, Hours.	Iodine Response	No.	Wt. Gms.	Time, Hours.	Iodine Response
11.a	650-900	48	Increase	3.d	835	24	Increase	17.h	650-900	48	Decrease
13.a	810	48	Decrease	5.e	1700	24	Increase	18.h	650-900	48	No ch'ge
14.a	810	48	Increase	6.e	1800	24	Increase	19.h	650-900	48	No ch'ge
22.b	810	48	Decrease	7.f	1056	24	Decrease	20.h	650-900	48	Decrease
26.b	810	48	Decrease	8.f	1015	48	Increase	21.h	650-900	48	Decrease
27.b	810	48	No ch'ge	9.f	942	48	Increase	23.h	650-900	48	Decrease
28.b	810	48	Decrease	10.f	942	48	Increase	38.i	768	48	No ch'ge
34.c	818	48	Increase	29.g	1234	48	Increase	39.i	510	48	Decrease
35.c	720	48	Decrease	30.g	1000	48	Increase	45.i	985	48	Decrease
37.c	572	48	Increase	31.g	975	48	Increase	46.i	850	48	No ch'ge
36.c	820	48	Decrease	32.g	679	48	Increase				
40.c	795	48	Decrease	33.g	700	48	Increase				
41.c	907	48	Decrease								
42.c	910	48	Decrease								
43.c	915	48	Decrease								
44.c	880	48	Decrease								
SUMMARY				SUMMARY				SUMMARY			
Total inc. iodine . . . . . 4				Total inc. iodine . . . . . 11				Total inc. iodine . . . . . 0			
Total no change . . . . . 1				Total no change . . . . . 0				Total no change . . . . . 4			
Total dec. iodine . . . . . 11				Total dec. iodine . . . . . 1				Total dec. iodine . . . . . 6			

The iodine may be so combined in the normal secretion of the gland as to form a physiological product, non-toxic in character, while in abnormal conditions, as in diseased thyroids or in the toxic desiccated glands, it may be split off into a smaller molecular grouping which may itself be toxic, or may be accompanied by a non-iodized substance which is responsible for the toxic effects. The list of possibilities is unlimited.

9. Brown, W. H.: Jour. Exper. Med., 1912, xv, No. 6.

To carry the analogy a step farther, the therapeutic value of official preparations is as a hematopoietic stimulant; correspondingly it is observed that in certain thyroid diseases iodine possesses a marked therapeutic value. As iron is for the most part found in hemoglobin, so is the greater part of the iodine found in the thyroid, doubtless as iodothyron or some closely related form. It is probable, then, that the increase of iodine in the colloid following artificial hyperthyroidism means an increase in organic iodine compounds of the thyroid.

Jones has shown that manipulative procedures, such as operations, and particularly shock, are conducive of a decrease of iodine. Yet this is an almost negligible factor, owing to the relatively slight trauma associated with a partial thyroid removal. The animals are usually sufficiently recovered within one-half hour to eat their food unconcernedly. The fact that our manipulative procedures effect no change in the colloid iodine has been controlled histologically.

Another factor which may have operated to cause a predominance in iodine loss in the normal series, is the fact that we have employed only adult rabbits for our source of normal and hypothyroid rabbit serum. Both adults and young were used in the production of a hyperthyroid serum and with the same result in both cases. If, as is generally supposed, the adult thyroid is normally less active than the young thyroid, then adult normal serum might be expected to be hypothyroid with respect to the plane of activity of the young rabbits employed as test objects. It would seem advisable in this connection to control by means of injections of serum of young rabbits into adult test animals.

If exophthalmic goiter is to be considered hyperthyroidism with an excess of an iodized thyroid secretion, we ought to be able to show it by an appropriate modification of our methods. Work along this line is now in progress.

#### SUMMARY

1. The relative iodine content of the thyroid colloid is determined by Mallory's connective tissue stain as applied by Jones.

2. The iodine content of the thyroid glands of rabbits serving as test animals was found to be increased directly by feeding commercial desiccated thyroids, and indirectly, by intravenous injections of serum from hyperthyroid rabbits.

3. The iodine content of the thyroid gland was found to be decreased by intravenous injections of serum of thyroidectomized rabbits.

4. Following normal serum injections, the iodine content was sometimes increased, sometimes decreased, or remained constant, indicating individual variations in thyroid activity.

The authors wish to express their appreciation of the kindly interest and criticism of Professors A. S. Loevenhart and C. H. Bunting.

## THE SOURCE OF URINARY INDOL-ACETIC ACID IN TWO DEMENTIA PRAECOX PATIENTS \*

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In this laboratory indol-acetic acid has been found in 21 per cent. of ninety-one urines of healthy normal persons.<sup>1</sup> Of 174 urines from dementia praecox patients 48 per cent. contained indol-acetic acid. The occurrence of the compound in the urine seemed to be related to a peculiar constitutional condition.

Hopkins and Cole<sup>2</sup> showed that *B. coli*, under partial aerobic conditions, would produce indol and indol-acetic acid from tryptophan. Salkowski<sup>3</sup> showed that if indol-acetic acid were given by the mouth, it immediately appeared in the urine. Herter<sup>4</sup> reported indol-acetic acid in the urine of an under-developed child suffering from an excessive and peculiar form of intestinal putrefaction. Putting these facts together, the natural conclusion would be that indol-acetic acid in the urine is a result of its production in the intestine by intestinal putrefaction of foods containing tryptophan. If this conclusion were true, indol-acetic acid, like indican, should be present in all urines, the quantity being dependent on the amount of intestinal putrefaction, and should be found in large quantities almost as frequently among sane as insane persons. In testing nearly 600 urines for indol-acetic acid over 50 per cent. gave no reaction for the compound.<sup>1</sup> A number of qualitative tests for indol-acetic acid and indican have not shown a high indican content to be always accompanied by a high indol-acetic acid content. So the following questions present themselves: (1) Is indol-acetic acid derived directly from ingested protein, and (2) is it derived from intestinal putrefaction, such as produces indican? In other words, is indol-acetic acid endogenous or exogenous?

### EXPERIMENTAL

Urorosein produced from indol-acetic acid in the urine by the action of hydrochloric acid and a small amount of an oxidizing agent, has a

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\* Preliminary Article from the Laboratory of the Illinois State Psychopathic Institute.

\* Submitted for publication June 23, 1913.

1. Ross: Preliminary Note on Excretion of Indol-Acetic Acid in the Urine. THE ARCHIVES INT. MED., 1913, xii.

2. Hopkins and Cole: Jour. Physiol., 1903, xxix, 451.

3. Salkowski: Ztschr. f. Physiol. Chem., ix, 26.

4. Herter: Jour. Biol. Chem., 1908, iv, 239.



bright red color. It was suggested to me by the director of the laboratory, Dr. H. Douglas Singer, that the intensity of the reaction might be measured with the Fleischl-Miescher hemoglobinometer. It was found that this could be done with a degree of exactness which compared very well with ordinary quantitative chemical analyses.

The next problem to solve was to evaluate the units of the scale of the hemoglobinometer in terms of indol-acetic acid. This was accomplished by extracting a large quantity of uroscopin from the urine with amyl alcohol. The amyl alcohol solution was treated with solid sodium hydroxid till the red color disappeared. The solution was filtered, and carbon dioxid passed through the filtrate till no more precipitate was formed. This solution was filtered and evaporated to dryness at room temperature. The residue was thoroughly washed repeatedly with ether, chloroform and xylene. The residue was then dissolved in ethyl alcohol, filtered and made up to a volume of 100 c.c. in a measuring flask. Measured volumes of the solution were run through the Kjeldahl method for nitrogen. Measured volumes were evaporated to dryness and the residue dissolved in measured volumes of amyl alcohol which had been saturated with HCl containing sufficient sodium nitrite to bring a maximum color. This red solution was then put into the 12 mm. chamber of the hemoglobinometer and the readings taken, using electric light as the luminant. A number of these determinations were done and the averages calculated. These averages and the empirical formula of indol-acetic acid, gave sufficient data to calculate the value of a single unit on the scale of the Fleischl-Miescher hemoglobinometer in terms of indol-acetic acid. The result was that each hemoglobinometer scale unit indicated .0788 milligrams of indol-acetic acid corresponding to the uroscopin in 1 c.c. of the amyl alcohol solution.

**Empirical Method of Determining Indol-Acetic Acid:** Twenty-five c.c. of urine were clarified with 5 c.c. of basic lead acetate<sup>5</sup> and filtered. Twenty c.c. of concentrated hydrochloric acid and 5 drops of 0.2 per cent. sodium nitrite were added and shaken for five minutes. The amyl alcohol was let out into a filter from which the hemoglobinometer chamber was filled. The Fleischl-Miescher hemoglobinometer with the 12 mm. chamber was used. An electric light was employed as the luminant. Using the scale unit value of the hemoglobinometer as determined above, the total indol-acetic acid in the urines collected each day was calculated. The whole process was carried out as rapidly as possible. Ten readings were made on each determination.

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5. The basic lead acetate was made up by the official methods of the chemists of the U. S. Department of Agriculture and then diluted with  $2\frac{1}{2}$  volumes of water.

I do not claim this method to determine absolutely all of the indol-acetic acid of the urine, nor the standardization of the scale units of the hemoglobinometer to be perfect. More work to this end is being done in this laboratory. It is, however, claimed that the method is accurate in determining the relative amounts in different urines. These points should be kept in mind in the consideration of the values given in the accompanying tables:

TABLE 1.—INDOL-ACETIC ACID AND INDICAN EXCRETION PER DAY

Date*	Patient M. P.		Patient M. O'B.	
	Indol-Acetic Acid in Milligrams	Indican in Milligrams	Indol-Acetic Acid in Milligrams	Indican in Milligrams
May 15 ....	.....	.....	.....	.....
16 ....	79.51	†	53.78	†
17 ....	78.45	.....	46.39	.....
18 ....	97.97	.....	64.75	.....
19 ....	161.11	16.88	97.36	11.90
20 ....	171.97	56.28	79.61	53.52
21 ....	121.52	47.36	86.97	35.51
22 ....	124.49	60.34	85.48	55.71
23 ....	.....	.....	.....	.....
24 ....	81.33	35.66	99.73§	17.76§
25 ....	111.97	30.07	76.53	18.85
26 ....	167.64	28.27	123.30	15.38
27 ....	198.25	8.41	119.47†	4.33†
28 ....	98.39†	25.64†	65.73	9.25
29 ....	97.46	38.70	57.73	19.18
30 ....	72.45	56.38	79.31	29.39
31 ....	102.40	46.44	98.48	26.75

\* Horizontal lines divide the periods. May 15 and 23 the losses were too great for determinations to be made.

† Not accurately determined.

‡ Indicates the loss of one or more urinations; in this instance the loss of one urine out of nine. These values are computed by multiplying the results

on the urine collected by the fraction:  $\frac{\text{Total urinations}}{\text{Total urinations collected}}$

§ Loss of two urines out of six.

The method of determining the amount of indican excreted was that of Ellinger as described by Dr. P. B. Hawk.<sup>6</sup>

6. Hawk, P. B.: Text-Book of Practical Physiological Chemistry, Edition 3, p. 387.

Determinations of indol-acetic and indican were made on each twenty-four-hour specimen as soon as collected. Duplicate determinations were made in each case.

The subjects of this test were two women patients in the Kankakee State Hospital. Both were classed in the dementia praecox group. Both patients at the time of the test seemed to be in perfect physical condition. Their body weights were nearly the same, each being between 100 and 110 pounds. During the test the patients were given a walk of nearly two miles each day.

TABLE 2.—INDOL-ACETIC ACID AND INDICAN EXCRETION PER DAY

Period	Patient M. P.		Patient M. O'B.	
	Indol-Acetic Acid in Milligrams	Indican in Milligrams	Indol-Acetic Acid in Milligrams	Indican in Milligrams
Tryptophan-free diet. Period I .....	104.26	Lost	65.57	Lost
High protein diet. Period II .....	139.33	54.66	84.02	48.25
Near protein-free diet. Period III ..	139.79	25.58	104.76	14.08
High protein diet. Period IV .....	92.67	41.79	75.31	21.14

The plan of the work was to determine the amounts of indol-acetic acid and indican excreted in the urine in twenty-four hours, while being nourished, first on tryptophan-free diet, second on a high protein diet, third on a non-protein diet, and fourth on high protein diet supplemented with agar to insure frequent bowel movements. The diet of the first period of four days consisted of corn-starch, tapioca, butterine, sugar, beef extract, gelatin, tea and coffee. The food of the second period of four days consisted of eggs, beefsteak, cheese in large amount, ham, oatmeal, a little potato, toast, coffee and tea. The food of the third period of four days was made up of corn-starch, tapioca, sugar, butterine, salt, very little beef extract, coffee and tea. The diet of the fourth period of five days consisted of eggs, beefsteak, oatmeal, potatoes (small amount), toast, coffee and tea. Roughly calculating the protein intake during the second and fourth periods it was at least 120 gm. per day, and for the third period not over 5 gm. per day per person.

The results of the analyses for indol-acetic acid and indican for the test are shown in Table 1.

It will be noted from the preceding table that it took one day after each change of diet for the indican output to be modified by the difference in diet. It is thought, therefore, that the results of the different diets will be most truthfully shown in the average of the daily output of the periods, including the first day after each change of diet in the preceding period. The results are as shown in Table 2.

The indican output was proportional to the protein ingestion, as would be expected. On the contrary, the indol-acetic acid output apparently did not vary directly with anything. It will be noted that the percentage of variation in the indol-acetic acid excretion was very small considering the extreme variations in diet. In view of this, it is suggestive that urinary indol-acetic acid excretion is more or less constant for the individual in a similar way to that of creatinin.

#### CONCLUSION

Urinary indol-acetic acid in the case of these two patients was endogenous.



## CULTURAL RESULTS IN HODGKIN'S DISEASE \*

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The publication at this time of the result of our endeavors to cultivate an organism from the tissues of patients with Hodgkin's disease is occasioned by the publication by Negri and Mieremet<sup>1</sup> of the successful cultivation from two cases of Hodgkin's disease of a diphtheroid organism which they designate as the *Corynebacterium granulomatis maligni*.

While Hodgkin's disease, or malignant granuloma of the lymphatic apparatus, has of late years come to be generally regarded as of infectious nature, the etiological agent has remained undetermined. The work in this country of Reed<sup>2</sup> and of Longcope<sup>3</sup> showed definitely that the condition was independent of tuberculosis, and that the theory supported by Sternberg<sup>4</sup> that it was a manifestation of the activity of the tubercle bacillus could not be maintained.

In 1900 Fraenkel and Much<sup>5</sup> reported that by treating Hodgkin's nodes with a strong alkalin solution of sodium hypochlorid (antiformin) they had found in the sediment, in twelve out of thirteen cases, certain granular, Gram-staining, but non-acid-fast bacilli, which they considered to be non-acid-fast tubercle bacilli, "possibly identical with the ordinary tubercle bacillus," but "more probably a special form of the tubercle virus," or at least "belonging to a related group of organisms."

In the present year Negri and Mieremet<sup>1</sup> have reported the successful cultivation on Bordet's medium (blood-glycerin-potato-agar), from two cases of Hodgkin's disease, of an organism which falls within the diphtheria group and further agrees in morphology with the forms described by Fraenkel and Much. The organism is a non-acid-fast, Gram-staining bacillus, growing luxuriantly at body temperature, and is a facultative anaerobe. Perhaps its most striking feature is its pleomorphism. The organism showed such variability in form that it was only after isolation of a single organism and cultivation of it that the authors were satisfied that they were dealing with a pure culture. In various media the follow-

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\* Submitted for publication June 5, 1913.

\* From the Pathological Laboratory of the University of Wisconsin.

\* Paper read before the Association of American Physicians, May 7, 1913.

\* This work has been aided by a grant from the Rockefeller Institute for Medical Research.

1. Negri and Mieremet: *Centralbl. f. Bakteriol.*, 1913, lxviii, 292.

2. Reed: *Johns Hopkins Hosp. Reps.*, x, 133.

3. Longcope: *Bull. Ayer Clin. Lab.*, 1903, No. 1.

4. Sternberg: *Ztschr. f. Heilk.*, 1898, xix, 21.

5. Fraenkel and Much: *Ztschr. f. Hyg.*, 1910, lxvii, 159.

ing forms were found: Plump, short rods, some so short as to resemble cocco-bacilli, these latter forms predominating or being exclusively found in old cultures; small thin bacilli with polar-staining; comma-shaped bacilli; granular rods of variable size, often of considerable length; branching forms; and on all media, club-shaped involution forms and large spherical forms. This organism is resistant to antiformin and the authors consider it identical with the organism described by Fraenkel and Much.

In a study of Hodgkin's disease, extending over the past five years, we have made various attempts to secure cultures from material removed at autopsy and at operation. The first attempts were made with ordinary media, and were unsuccessful. In Case 9 of our reported series,<sup>6</sup> cultures were made at operation, in October, 1910, by planting portions of lymph-nodes in bouillon, but apparently in this case there was a complicating infection, as only a streptococcus growth was obtained. In Case 5 of the series, attempts were made to secure cultures from the enlarged mesenteric nodes at autopsy, in January, 1912, but post mortem invading organisms completely overgrew the media.

After these failures, it was felt that special media must be used, and as at this time we felt from certain observations that the organism was probably one of the higher forms of bacteria, if not a fungus, media appropriate to the growth of these organisms was selected. Dorset's egg-medium and glycerin-phosphate-agar were first tried. The first opportunity for their use came in an operation by one of us (J. L. Yates) for the removal, on Feb. 13, 1912, from a woman patient, of a large group of cervical lymph-nodes.

The essential points in her history are as follows:

CASE 1.—Female, white, age 33. When first seen in October, 1911, the patient showed in the right side of her neck a group of enlarged, discrete, lymph-nodes extending from the mid-clavicular region upward in the posterior triangle practically to the attachment of the sternocleido-mastoid. This group of nodes had shown progressive enlargement during the year previous. Except for one small node below the clavicle on the right side and a rather large gland in the right axilla, no further enlargement of lymph-nodes was noted at this time. The spleen was not palpable. The tonsils were enlarged. October 10, a von Pirquet test and a Wassermann reaction were both negative. October 23 the large axillary node was removed for diagnosis. In section it showed hyperplasia of lymphoid and of endothelial elements with giant cells of the endothelial type and of the megalokaryocyte type, disappearance of the architecture of the node, and a beginning diffuse sclerosis and marked eosinophilic infiltration. December 11 tonsillectomy was done, and Feb. 13, 1912, the large group of lymph-nodes in the right side of the neck with the intervening sclerotic tissue, was removed en masse.

After removal the group of nodes was incised, and, with the observation of the strictest aseptic technic, portions of the nodes and of the interglandular tissue were planted on the surface of slants of the egg-medium and of the glycerin-

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6. Bull. Johns Hopkins Hosp., 1911, xxii, 368.

phosphate-agar. The tubes were sealed with paraffin and incubated at 37 C. The tubes were apparently sterile at the end of ten days' incubation, but they were left undisturbed in the incubator, and at the end of three weeks' time a slight growth was noted on one tube. March 12, two of the egg tubes and one tube of glycerin-phosphate-agar showed pure cultures of a diphtheroid organism, with many clubbed-shaped involution forms, with granular and barred rods and evidently branching filaments. The organism was Gram-staining.

Subcultures were made from the original tubes on blood-serum, egg, glycerin, agar, and the ordinary media. The organism proved to be a rather feeble grower under the conditions under which its growth was attempted at that time. On blood serum in twenty-four hours there were obtained the characteristic shining, opaque, white colonies. On agar the growth was slow, the streak at first being of a grayish, glistening, translucent appearance, becoming more opaque with age. In bouillon there was a scanty growth which gradually settled to the bottom as a somewhat slimy precipitate. No turbidity of the medium occurred. Milk was not coagulated. Its reaction was changed but little if any.

The growth was tested on guinea-pigs, and while the results were unsatisfactory in a sense, it was determined that the animals would survive inoculation with a heavy dosage of a twenty-four-hour culture for ten days at least. Through an accident this culture was lost before thorough testing of it could be done.

Further opportunities for obtaining cultures were not obtained until January, 1913, when, through the kindness of Dr. W. J. Mayo and the cooperation of his staff, the courtesies of the clinic at Rochester were extended to us.

On Jan. 9, 1913, cultures were made from a cervical gland removed for diagnostic purposes from a patient with the following history:

CASE. I.—Female, white, aged 34 years. Four years previously she noticed a swelling on the right side of her neck, followed by the appearance of other small tumors. Ten months before date enlarged lymph-nodes appeared on the left side of the neck and in the right axilla. Six weeks previously nodes appeared in the left axilla. Physical examination revealed, in addition to these nodules, a mediastinal mass, a six months' pregnancy, over which were felt discrete firm nodules which slipped under the examining hand. Nose and throat examination revealed a hypertrophic rhinitis, causing some nasal obstruction and also enlarged tonsils. Red blood-cells, 4,480,000; leukocytes, 12,400.

Pathology of Node.—The test node shows microscopically loss of architecture, with lymphoid and endothelial hyperplasia, the presence of many endothelial giant cells, a beginning fine sclerosis and eosinophilic infiltration. There is slight invasion of the capsule.

Pieces of the gland were planted on egg-medium and on Loeffler's blood-serum. The tubes were sealed and incubated. On January 17 one egg-tube showed the characteristic clubbed and granular diphtheroid forms seen in the first culture; but associated with a growth of *B. subtilis*. One serum-tube showed a pure culture of the diphtheroid organism, and one serum tube showed the presence of a white staphylococcus. Three other tubes were sterile.

Subcultures of the diphtheroid organism were made from the serum tube on to other media, particularly egg-medium, glycerin-phosphate-agar and plain agar. The growth proved to be luxuriant on these. The top of the streaks had a grayish glistening appearance, while the lower part of the streaks were at first of an opaque white appearance, gradually assuming a slight yellowish color, deepening in very old cultures, as glycerin-agar, to a brownish. Smears from the older cultures showed such a pleomorphism, and there were so many coccoid forms it was at first thought we were dealing with a mixed culture of bacillus and coccus. It was only after growth of many generations on egg-medium, on which the culture is of a pure bacillary type during the first twenty-four hours, and after shaking the culture with sand and plating from a



further dilution, which gave colonies of a uniform and characteristic type, that we became assured we were dealing not with a symbiosis of coccus and bacillus, but with a pleomorphic organism.

A third successful cultivation was made Feb. 15, 1913, from a cervical lymph-node from the following case from the Mayo Clinic:

CASE 3.—Married female, white, aged 38, with marked enlargement of the cervical nodes and rather general glandular enlargement. The illness began with involvement of the cervical glands two years previous to date.

The node removed for diagnosis showed extreme sclerosis, but with scattered alveoli-like areas in which the characteristic elements of the Hodgkin's lymph-node, the endothelial cells, giant cells and lymphocytes were preserved. Eosinophilic infiltration was well marked.

After one week's cultivation there were secured from two tubes, one of Loeffler's serum and the other of egg-medium, a pure culture of a diphtheroid, pleomorphic organism apparently identical with that secured from the previous case, showing the same cultural characteristics. It was put through the same procedure to determine the purity of the culture and gave the same result, that is, after shaking with sand and diluting and plating, all the colonies on the plates were of a uniform character.

In the interval between the last two cases, attempts were made to secure cultures from two other cases, but they resulted less successfully. The first of these attempts was at a post-mortem, Jan. 15, 1913, on the following case:

CASE 4.—Female, white, aged 50 years (patient of Dr. Reeves of Albany, Wis.). The glandular swelling had first appeared in the left cervical region two years before. The cervical group of lymph-nodes had been removed about six months previous to the patient's death. At the post-mortem examination there was found rather general glandular involvement, but especial enlargement of the mesenteric and retroperitoneal nodes and of the lymphoid elements of the spleen. There was also a chylous ascites, well marked anemia and emaciation. Old pleural scars were noted at the pulmonary apices and one calcified focus in a bronchial lymph-node. No other signs of tuberculosis were seen.

Microscopically, the abdominal lymph-nodes showed a well-marked hyperplastic Hodgkin's disease picture with beginning diffuse sclerosis, while the splenic lesions showed a more advanced sclerotic condition.

On one Loeffler serum-tube, in which was planted a piece removed from the interior of the spleen, there was noted a growth on the tissue itself of typical polar-staining diphtheroid organisms, but for some reason unascertained, they would not "catch on" to the medium, and attempted subcultures from scrapings of the tissue on both the egg-medium and on serum proved negative.

The second case was the following from the Mayo Clinic, Jan. 31, 1913:

CASE 5.—Male, white, aged 33. The patient had had an abscess of a tooth six months previously. About three weeks before coming to the hospital patient had noticed a painless swollen node in the left supraclavicular region. This had increased in size during the time of observation. The large node removed was of a uniform medullary appearance on gross section, and microscopically showed lymphoid and endothelial hyperplasia, with the presence of giant cells, beginning diffuse sclerosis and eosinophilic infiltration. The architecture of the node was destroyed.

Unfortunately for the success of cultural investigation, this gland had been handled and incised before the plants on the media were made. Four days after



incubation of the tubes was begun, there was found on one of the serum-tubes a growth of the typical diphtheroid organism, but in association with a coarse bacillus of the *B. subtilis* type. As at this time our efforts were being directed to the elimination of the supposed coccoid contamination of our second culture, no attempt was made to separate the diphtheroid organism from this last mixed culture.

While we have made no systematic attempt to find the organism described in histological preparations from the nodes of Hodgkin's disease patients, one case should be recorded as of interest in connection with the cultural results. Portions of the organs from a girl of 6 years, a patient of Dr. Ogden of Milwaukee, who died in a severe vomiting attack, were received in the laboratory. The Peyer's patches of the intestine and the mesenteric lymph-nodes showed the changes of early Hodgkin's disease, which from clinical and post mortem evidence must have been primarily intestinal in origin. On staining the sections by the Gram-Weigert method, the only organisms found, save for the surface intestinal bacteria, were groups of polar- and granular-staining diphtheroid organisms lying deep within the enlarged Peyer's patches.

#### SUMMARY OF CULTURES

To summarize our cultural results: In three cases of Hodgkin's disease we have secured a pure culture of a pleomorphic diphtheroid organism. In two other cultural attempts the organism was recognized, but was not secured in pure culture, and in a sixth case a similar organism, morphologically, was stained in the lesions of a primary intestinal Hogkin's case.

The detailed biological reactions of this diphtheroid organism have as yet not been completely worked out by us. The strain recovered from the second case, however, has been found to grow readily at 37 C. on the media used to secure the cultures and on ordinary agar-agar. On glycerin-phosphate-agar the growth is almost as luxuriant under strict anaerobic as under aerobic conditions. For luxuriant growth, marked moisture of the medium seems necessary. On a relatively dry medium, growth is slow, and the organisms are found to develop as the long forms, granular, banded, and with many club-shaped involution forms. Branching forms are also noted. These forms are especially well developed on the egg-medium, where they also seem to have a tendency to cohere, so that in stained smears one gets many small groups of organisms radially arranged, with clubbed peripheral elements, somewhat suggestive of a minute actinomyces colony, as seen in section. On moist serum tubes with luxuriant growth, the organisms are short and plump, with polar staining. Many of these forms are coccoid. We have noted also,

as emphasized by Negri and Mieremet, that in all old cultures coccoid forms predominate, and also that large spherical involution forms are present. A colony or a streak, which at twenty-four hours shows entirely the bacillary form, will twenty-four or forty-eight hours later show an apparent outnumbering of the bacilli by the coccoid elements.

The organism stains by the Gram method, though the short plump forms hold the dye less strongly than the longer bacillary forms. It is not acid fast. No spore formation has been noted.

The growth of the organism is at first glistening and grayish, but becomes more opaque and of a white color. Apparently, depending somewhat on the reaction of the media, there may be in some early cultures a slight greenish-yellow tint produced. Old cultures on glycerin-phosphate-agar become brownish, and the media itself darkens.

Gelatin is not liquefied. There is no early change in reaction in litmus milk. Bouillon is not clouded by the growth. Flecks appear along the side of the tube, and a slimy deposit gradually accumulates at the bottom.

Plate cultures show a rounded colony with quite regular edge, a fine stippling of the growth and a central dark spot. The colonies are of a glistening gray color at the end of twenty-four hours, becoming gradually of an opaque white color.

Altogether, our studies thus far seem to indicate that we are dealing with the same organism described by Negri and Mieremet, and while our results taken with theirs cannot be said to indicate positively that we are dealing with the cause of Hodgkin's disease, yet they are strongly suggestive. The morphological elements obtained by Fraenkel and Much by the antiformin method in twelve out of thirteen cases are so similar to the organism cultivated by us that we find added support in their findings to the importance of the organism in the disease. Ultimate proof can come only from an extended series of cultures, or from animal experimentation. The latter test is now in progress in this laboratory with a variety of animal species. Should the relation of the organism to the disease be established, we would suggest *Corynebacterium Hodgkini* as a more appropriate name for the species than the trinomial designation suggested by Negri and Mieremet.

Although the utmost efforts were made to prevent carrying in organisms from the skin, when removing nodes for cultural investigation, in almost every case studied, one or more tubes have shown the presence of a white staphylococcus. This, when taken in connection with the polymorphonuclear leukocytosis, which occurs late in the disease, has suggested that possibly a secondary infection plays a part in the development of the disease. This feature also needs further investigation.

## ADDENDUM

After this paper was placed in the hands of the publishers, the opportunity presented to make cultures in another case of Hodgkin's disease from a patient in the charge of Dr. Frank Billings of Chicago. The essential points in the patient's history are as follows:

Male, white, aged 32. Loss of weight since Jan. 1, 1913. During a period of six weeks before date of examination (April 23, 1913) gradual enlargement of the nodes in the neck and groin and to some extent in the axillae was noted. On examination there was found moderate enlargement of the tonsils, cervical, axillary and inguinal nodes and a firm, palpable spleen.

April 28, blood-count was, R. B. C., 4,150,000; W. B. C., 4,150. Hgb., 70 per cent. (Dare). April 28 a node from the left side of the neck was removed by Dr. Dean Lewis. This showed on section endothelial proliferation with endothelial giant cells, some fine diffuse sclerosis and but little eosinophilic infiltration.

May 7, Dr. F. B. Moorehead extracted right upper first molar and second bicuspid teeth, finding a large amount of granulation tissue around the roots, and along the lingual root of the molar an abscess cavity about 1 cm. in length containing pus of foul odor. Unfortunately, no cultures were made.

May 29, with the observation of the strictest precautions to prevent contamination, Dr. Lewis removed a node from the left cervical region, and one from the right inguinal region.

In the laboratory of Dr. E. C. Rosenow, and with his assistance, which is gratefully acknowledged, pieces of these nodes were planted on Loeffler's serum, egg-medium, blood-agar and serum-glucose-agar. Other portions of the nodes were ground in a sterile mortar and the emulsion planted on tubes of the media mentioned.

From these plants growth of the diphtheroid organism was obtained in pure culture on Loeffler's serum from both the cervical and the inguinal nodes where solid pieces of the node were used. The organism was also obtained from the cervical node, where the emulsion was planted, but with a contaminating pigment-producing air organism.

Dr. Rosenow reports positive results from the cervical node on blood-agar slants, aerobic and anaerobic, and from the depths of one tube of serum-glucose-agar. From the inguinal node two colonies developed in the depths of the serum-glucose-agar tube.

## BOOK REVIEW

**DIGESTION AND METABOLISM.** The Physiological and Pathological Chemistry of Nutrition. For students and physicians. By Alonzo Englebert Taylor, M.D., Rush Professor of Physiological Chemistry, University of Pennsylvania, Philadelphia. Octavo, 560 pages. Cloth, \$3.75 net. Lea & Febiger, Philadelphia and New York, 1912.

The medical profession of America owes Dr. Taylor a debt of gratitude for making available to those who read only English the vast extent of chemical knowledge of the processes of digestion and nutrition which he has put between the covers of his book. The general plan of the work, the sequence of the chapters, the completeness with which he has treated each subject, and the vigorous and positive style which Dr. Taylor always has at his command, all contribute to make it a noteworthy text-book. To the reviewer the systematic use of full chemical formulas throughout the book seems especially commendable. They conduce to visualization of the actual chemical processes described and, therefore, educate the reader to think chemically, a much needed ability for the modern physician.

With so much that is admirable to praise, it seems ungracious to ask for more; yet the reviewer cannot but regret profoundly that Dr. Taylor was content to produce only a text-book. That he did this advisedly, he says in his preface, and that after one-third of the work was completed as a book of reference. Only then did he determine to omit all statement of his authorities and his bibliography. Had he continued as he began, the book would have been of the utmost value to American medical science, which is becoming especially strong in the field of physiological chemistry, and would have supplemented in a welcome manner Lusk's *Science of Nutrition*. Only in America would a man of Professor Taylor's learning think of producing a work of this magnitude in the form of a student's text-book. It is far too exhaustive and detailed to meet the need only of learners, and it is to be hoped that Professor Taylor may be persuaded to add his references in a future edition, in order that the usefulness of his work may extend to his fellow-investigators as well. Even for the American student, separated as he is from contact with foreign investigators, it is most desirable that he should become familiar with their names and their contributions to the knowledge which is handed on to him for use.

In certain details the lack of citation of authority is particularly felt. On pp. 239-240, in a rather inadequate discussion of the formation of sugar from amino-acid, this is true. The statement that the human diabetic gets two or three times the amount of sugar from protein that the normal body does, cannot be accepted without a fuller discussion. On page 259 one finds this statement: "The ingested sugar must in these cases be burned, if resorbed, since there is no glucosuria and, therefore, no hyperglucemia. Typical instances of acute exophthalmic goiter seems to behave in the same way." As a matter of fact, the investigation of the blood sugar in exophthalmic goiter is revealing hyperglucemia with great frequency. The reviewer would have been much indebted to Professor Taylor for his references to these typical acute cases. On page 264 he states: "We know that when the pancreas is removed the muscles cannot burn glucose properly. This positive fact is far more fundamental than the negative results of test-tube experiments with extracts of muscle and pancreas." Unfortunately, there is at present much uncertainty about this fundamental "positive fact," which has recently been denied by von Noorden. Though few would agree with the latter's views, they do not make definite statement desirable, without full discussion and references to the sources of knowledge on the subject. Also, on



page 271, reference is made to the presence of acetone in the blood. This is usually denied.

However, the whole discussion of the combustion of glucose is written in a most interesting way, as is the chapter on ferments, Professor Taylor's special interest. The present state of knowledge in each field is well presented, and the information, as far as can be judged without a statement of sources, is accurate. No one, student or investigator, can read the book without real pleasure and great profit, and it is to be hoped that many will be induced to. Some errors demand correction in the next printing. On page 47, "arginin" is spelled "argenin." On page 248 the equation "glucose + water = glycogen" is reversed. On page 265 "intestinal secretion" should be "internal secretion." Professor Taylor also coins some words to which the reviewer objects, especially "input" for intake. But these are insignificant criticisms of a book which has been read with unusual interest.

# The Archives of Internal Medicine

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Vol. XII

SEPTEMBER, 1913

No. 3

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## THE RELATION BETWEEN NON-PROTEIN NITROGEN RETENTION AND PHENOLSULPHONEPHTHALEIN EXCRETION IN EXPERIMENTAL URANIUM NEPHRITIS \*

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One of the important problems in connection with the study of nephritis is the determination of the ability of the kidneys to remove the metabolism products which normally find their way into the urine. Three different lines of investigation have been pursued in the study of this problem, namely, (a) urine analysis, the direct determination of urinary constituents, nitrogen, urea, chlorids, etc., (b) blood analysis, the determination of similar products in the blood, particularly the so-called uncoagulable nitrogen and the urea, (c) the determination of the ability of the kidney to eliminate certain foreign substances, such as potassium iodid or dyes, indigo-carmin, rosanilin, phenolsulphone-phthalein, etc.

The first of these lines of investigation, urine analysis, has contributed very little, except in relation to the elimination of chlorids and water. To anyone familiar with the uncertainties and variations as well as the difficulties involved in nitrogen equilibrium experiments, this lack of definite results is by no means strange.

Investigation of the nitrogenous products retained in the blood in nephritis seemed from the beginning the most direct as well as the most promising point of attack, except for the great difficulties involved in determining the nitrogenous waste products in such a complex and highly nitrogenous fluid as blood. Accordingly, very little exact information is as yet available with regard to the relation between the accumulation

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\*Submitted for publication April 14, 1913.

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of waste nitrogen in the blood and various stages and kinds of nephritis, although the literature on the subject is very voluminous.<sup>1</sup>

Studies of renal efficiency by means of various foreign substances, particularly dyes and indicators, have one great advantage in that they offer an almost unlimited scope for selection with reference to harmlessness, ease of estimation and the readiness with which the substances are eliminated by the kidneys. The chief drawback attached to this line of work lies in the interpretation of the results obtained, for it is quite conceivable that the rate with which the kidneys eliminate any one such foreign substance may have no definite relationship to the efficiency of the kidneys with reference to metabolism products. By far the most serviceable substance as yet introduced in this field is phenolsulphonephthalein.<sup>2</sup> In this paper will be found a review of the earlier literature on the subject. The numerous observations recorded by Rowntree and his associates on the speed with which phenolsulphonephthalein is eliminated by normal as well as by nephritic kidneys seem to show that the elimination of this substance when injected intramuscularly is indeed a valuable index to the general efficiency of the kidneys. The observations have been confirmed by several other investigators.<sup>3</sup>

The desirability of correlating the elimination of this substance with the actual retention in the blood of nitrogenous waste products is self-evident. Rowntree and Fitz<sup>4</sup> have already attempted such correlation, but so long as no reliable method existed for the determination of the urea and non-protein nitrogen in the blood the results obtained must necessarily remain obscure and uncertain. By means of the new methods of Folin and Denis<sup>5</sup> it is possible to determine with the necessary accuracy both the total non-protein nitrogen and urea in the blood.<sup>6</sup> These methods have the added advantage that only small quantities of blood (2 to 5 c.c.) are needed, thus making possible a continuous study of experimental nephritis in ordinary small laboratory animals, such as cats and rabbits.

For the work described in this paper we have used rabbits in whom acute nephritis was produced by means of a single dose of uranium

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1. Strauss: die chronischen Nierenentzündungen, 1902; Widal: Compt. rend. Soc. de biol., 1904, p. 301; Obermayer and Popper: Ztschr. f. klin. Med., 1911, lxxii, 332; Hohlweg: Deutsch. Arch. f. klin. Med., 1911, civ, 216; Nellis Foster: THE ARCHIVES INT. MED., 1912, x, 414; Folin, Karsner and Denis: Jour. Exper. Med., 1912, xvi, 789.

2. Rowntree and Geraghty: Jour. Pharmacol. and Exper. Therap., 1910, i, 579.

3. Sehrl: Zentrbl. f. Chir., 1912, No. 33, p. 1121; Deutsch. Wien. klin. Wehnschr., 1912, No. 32, p. 1217; Autenrieth and Funk: München. med. Wehnschr., 1912, No. 49, p. 2657; Erne: München. med. Wehnschr., 1913, No. 10, p. 510; Eisenbrey: Jour. Exper. Med., 1911, xiv, 462.

4. Rowntree and Fitz: THE ARCHIVES INT. MED., 1913, xi, 258.

5. Folin and Denis: Jour. Biol. Chem., 1912, xi, 527.

6. Folin and Dennis: Jour. Biol. Chem., 1913, xiv, 31.

nitrate (from 1.25 to 3 mg.) given subcutaneously. Two series of experiments were made. In the first series the animals were killed (under anesthesia) by bleeding from the carotid arteries. They were killed on consecutive days from one to ten days after the administration of the uranium nitrate in order to secure histological record of the pathological lesions in the kidney, and to determine their relation to the non-protein nitrogen in the blood and the urinary tests at different stages of the nephritis. In these experiments the blood was analyzed only on the day that the animals were killed. In the second series of experiments the animals were allowed to recover, and blood analyses as well as phenolsulphonephthalein tests were made periodically. The blood in these experiments was obtained from the veins of the ear. Because of the small quantity of blood taken each time (2 to 3 c.c.) no reduction in the hemoglobin of the animals was observed as a result of the frequent bleeding.

The rabbits were kept in cages of moderate size so that they could move around easily. They were fed with carrots and hay, and except for a few days at the height of the nephritis, each rabbit consumed about 100 grams of carrot per day. Just before the injection of the phenolsulphonephthalein solution (1 c.c. containing 6 mg. of the indicator) into the muscles of the thigh each rabbit was given 50 c.c. of water by means of a stomach tube. The animal was kept in a small cage over a glass funnel, to prevent loss of urine, for seventy minutes and the bladder was then emptied by means of massage. One-half of the urine so obtained was diluted to 500 c.c., rendered alkaline and its phenolsulphonephthalein content was determined colorimetrically, using 1 c.c. of the original phenolsulphonephthalein solution, diluted to one liter, as a standard. The determination was made exactly as described by Rowntree and Geraghty,<sup>7</sup> using their modification of the Autenrieth-Königsberger colorimeter. The other half of the urine was used in connection with the examination for casts and tests for albumin. Nitric acid was used throughout for the albumin tests.

The kidney tissues were preserved for the histological examination (a) in Zenker's solution, (b) in formalin (10 per cent.), and were then stained (a) with eosin and methylene blue and (b) with scharlach R.

The blood of normal rabbits contains about 30 mg. of total non-protein nitrogen and about 13 mg. of urea nitrogen per 100 gm.<sup>7</sup> The phenolsulphonephthalein excretion in such rabbits amounts to 60 per cent. or over in seventy minutes when 6 milligrams are injected under the described conditions.

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7. Rowntree and Geraghty: THE ARCHIVES INT. MED., 1912, ix, 284.



## EXPERIMENTS

*Experiment 1.*—Rabbit 518.<sup>s</sup> Urine normal. Died<sup>o</sup> on the *first day* after the administration of the uranium nitrate. (Acute pulmonary edema.)

First day (wt. 1,520 gm.): Phenolsulphonephthalein excretion.....70 per cent.

Administered 3 mg. uranium nitrate.

Second day (wt. 1,535 gm.): Phenolsulphonephthalein excretion....30 per cent.

Urine: albumin trace; epithelial cells but no casts.

Blood: non-protein nitrogen (per 100 gm.).....41 mg.

Blood: urea nitrogen (per 100 gm.).....13 mg.

Histological Findings: Glomeruli slightly congested. Tubules negative; no excess of fat.

*Experiment 2.*—Rabbit 520. Urine normal. Killed on the *second day* after the administration of uranium nitrate.

First day (wt. 1,140 gm.): Phenolsulphonephthalein excretion....54 per cent.

Administered 3 mg. uranium nitrate.

Second day (wt. 1,140 gm.): Phenolsulphonephthalein excretion....46 per cent.

Urine: albumin trace; epithelial cells present; no casts.

Third day (wt. 1,140 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin trace; epithelial cells and a few casts.

Blood: non-protein nitrogen (per 100 gm.).....32 mg.

Blood: urea nitrogen (per 100 gm.).....13 mg.

Histological Findings: Glomeruli slightly congested. Necrosis with desquamation of epithelium in some tubules. No excess of fat. A few areas of round cell infiltration.

*Experiment 3.*—Rabbit 521. Faint trace of albumin in urine, no casts. Killed on the *third day* after the administration of the uranium nitrate.

First day (wt. 1,350 gm.): Phenolsulphonephthalein excretion....68 per cent.

Administered 3 mg. uranium nitrate.

Second day (wt. 1,300 gm.): Phenolsulphonephthalein excretion....31 per cent.

Urine: albumin faint trace; epithelial cells; no casts.

Third day (wt. 1,210 gm.): Phenolsulphonephthalein excretion....20 per cent.

Urine: albumin trace; epithelial cells; no casts.

Fourth day (wt. 1,160 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin trace; epithelial cells; casts.

Blood: non-protein nitrogen (per 100 gm.).....116 mg.

Blood: urea nitrogen (per 100 gm.).....69 mg.

Histological Findings: Glomeruli negative; considerable necrosis and desquamation of certain tubules; necrotic material in lumen of other tubules.

*Experiment 4.*—Rabbit 523. Urine normal. Killed on the *fourth day* after the administration of the uranium nitrate.

First day (wt. 1,400 gm.): Phenolsulphonephthalein excretion....75 per cent.

Administered 3 mg. uranium nitrate.

Second day (wt. 1,390 gm.): Phenolsulphonephthalein excretion....58 per cent.

Urine: albumin trace; epithelial cells present; no casts.

Third day (wt. 1,330 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin trace; epithelial cells present; no casts.

Fourth day (wt. 1,370 gm.): Phenolsulphonephthalein excretion.....trace

Urine: practically anuric.

Fifth day (wt. 1,370 gm.): Phenolsulphonephthalein excretion.....trace

Urine: no albumin; many casts.

Blood: non-protein nitrogen (per 100 gm.).....142 mg.

Blood: urea nitrogen (per 100 gm.).....109 mg.

8. In the following experiments the animal numbers are those recorded in the laboratory of Theory and Practice.

9. Blood had not clotted and was collected from the right pleural cavity.

Histological Findings: Glomeruli negative. Necrotic epithelium in many tubules; disappearance of epithelium in certain tubules; necrotic material and casts in others. Slight excess of fat in necrotic material. A few areas of round cell infiltration.

*Experiment 5.*—Rabbit 524. Urine normal. Found dead on the morning of the *fifth day* after the administration of uranium. Blood<sup>10</sup> taken from the dead animal for analysis.

First day (wt. 1,440 gm.): Phenolsulphonephthalein excretion....68 per cent.

Administered 3 mg. uranium nitrate.

Second day (wt. 1,425 gm.): Phenolsulphonephthalein excretion...45 per cent.

Urine: albumin trace; epithelial cells present; no casts.

Third day (wt. 1,400 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin large trace; epithelial cells present; few casts.

Fourth day (wt. 1,400 gm.): Phenolsulphonephthalein excretion.....trace

Urine: practically anuric.

Fifth day (wt. 1,380 gm.): Phenolsulphonephthalein excretion.....anuric

Sixth day: Blood: non-protein nitrogen (per 100 gm.).....256 mg.

Blood: urea nitrogen (per 100 gm.).....172 mg.

Histological Findings: Edema and congestion of glomeruli. Necrotic epithelium in many tubules; disappearance of epithelium in many; necrotic material and casts in others. Slight excess of fat in necrotic material and tubular epithelium. No round cell infiltration.

*Experiment 6.*—Rabbit 532. Urine normal. Killed on the *fifth day* after the administration of the uranium.

First day (wt. 1,260 gm.): Phenolsulphonephthalein excretion....68 per cent.

Administered 3 mg. uranium nitrate.

Fourth day (wt. 1,175 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin large trace; many casts.

Sixth day (wt. 1,225 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin trace; a few casts.

Blood: non-protein nitrogen (per 100 gm.).....216 mg.

Blood: urea nitrogen (per 100 gm.).....125 (?) mg.

Histological Findings: Glomeruli negative. Disappearance of epithelium in certain tubules; regeneration of epithelium in others; necrotic material and casts in some. A few areas of round cell infiltration.

*Experiment 7.*—Rabbit 528. Urine normal. Killed on the *seventh day* after the administration of the uranium.

First day (wt. 1,750 gm.): Phenolsulphonephthalein excretion....79 per cent.

Administered 3 mg. uranium nitrate.

Second day (wt. 1,720 gm.): Phenolsulphonephthalein excretion....49 per cent.

Urine: albumin trace; epithelial cells present; no casts.

Third day (wt. 1,660 gm.): Phenolsulphonephthalein excretion....19 per cent.

Urine: albumin large trace; epithelial cells present; a few casts.

Fourth day (wt. 1,660 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin trace; few casts.

Fifth day (wt. 1,620 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin trace; many casts.

Sixth day (wt. 1,500 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin trace; many casts.

Seventh day (wt. 1,470 gm.): Phenolsulphonephthalein excretion...10 per cent.

Urine: albumin slight trace; many casts.

Eighth day (wt. 1,460 gm.): Phenolsulphonephthalein excretion...38 per cent.

Urine: albumin negative; many casts.

Blood: non-protein nitrogen (per 100 gm.).....92 mg.

Blood: urea nitrogen (per 100 gm.).....62 mg.

10. Blood had not clotted and was taken from right pleural cavity.

**Histological Findings:** Glomeruli negative. Necrotic epithelium in a few tubules; considerable regeneration in some tubules. Necrotic material and casts in collecting tubules. Fat in necrotic material and in the epithelium of some tubules. Round cell infiltration in many areas.

*Experiment 8.*—Rabbit 529. Urine normal. Killed on the *eighth day* after the administration of uranium nitrate.

First day (wt. 1,380 gm.): Phenolsulphonephthalein excretion....72 per cent.

Administered 3 mg. uranium nitrate.

Second day: Phenolsulphonephthalein excretion.....59 per cent.

Urine: albumin slight trace; no casts; epithelium present.

Third day (wt. 1,310 gm.): Phenolsulphonephthalein excretion.....2 per cent.

Urine: albumin trace; epithelial cells present; a few casts.

Fourth day (wt. 1,310 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin large trace; epithelial cells; rare casts.

Fifth day (wt. 1,340 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin large trace; many casts.

Sixth day (wt. 1,360 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin none; few casts.

Seventh day (wt. 1,260 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin slight trace; many casts.

Eighth day (wt. 1,230 gm.): Phenolsulphonephthalein excretion....14 per cent.

Urine: albumin slight trace; many casts.

Ninth day (wt. 970 gm.): Phenolsulphonephthalein excretion.....12 per cent.

Urine: albumin negative; many casts.

Blood: non-protein nitrogen (per 100 gm.).....266 mg.

Blood: urea nitrogen (per 100 gm.).....200 mg.

**Histological Findings:** A few glomeruli show hyaline droplets. Considerable necrotic epithelium in tubules; regeneration in some. Necrotic material and casts in tubules. No excess of fat. No round cell infiltration.

*Experiment 9.*—Rabbit 533. Urine normal. Killed on the *tenth day* after the administration of uranium nitrate.

First day (wt. 1,180 gm.): Phenolsulphonephthalein excretion....66 per cent.

Administered 2 mg. uranium nitrate.

Fourth day (wt. 1,125 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin trace; epithelial cells and some casts present.

Sixth day (wt. 1,080 gm.): Phenolsulphonephthalein excretion.....1 per cent.

Urine: albumin trace; many casts.

Eighth day (wt. 1,010 gm.): Phenolsulphonephthalein excretion....42 per cent.

Urine: albumin slight trace; many casts.

Ninth day (wt. 975 gm.): Phenolsulphonephthalein excretion.....34 per cent.

Urine: no albumin; a few casts.

Eleventh day (wt 910 gm.): Phenolsulphonephthalein excretion....60 per cent.

Urine: normal.

Blood: non-protein nitrogen (per 100 gm.).....59 mg.

Blood: urea nitrogen (per 100 gm.).....43 mg.

**Histological Findings:** Glomeruli negative. Tubules negative except for a few showing regeneration. No excess of fat. Numerous areas of round cell infiltration.

From Experiments 1 to 9, recorded above, it is evident that the excretion of phenolsulphonephthalein is very much diminished on the day following the administration of uranium nitrate, and that by the second or third day the excretion is reduced to a mere trace or ceases altogether. The accumulation of the nitrogenous waste products in the blood is of course gradual, and would naturally be very slow in a herbiv-

orous animal living chiefly on carrots. After the third day, however, the accumulation of non-protein nitrogen and urea in the blood reaches extraordinary figures. The fact that the animals often abstained from eating during this period probably resulted in their using up of their supply of glycogen, and this would greatly accelerate the protein destruction and the accumulation of the nitrogenous products. Variations in the accumulation during the anuric period are doubtless chiefly due to variations in the consumption of carbohydrates. As the retention of nitrogen increases the proportion represented by the urea becomes increasingly larger. The histological evidence of injury to the kidney from the uranium did not appear until the second day after the administration of the uranium. Traces of albumin in the urine appeared, on the other hand, uniformly at the end of the first day, though casts did not occur until the third day. After the third day, when extensive necrosis of the tubular epithelium is found, the enormous accumulation of nitrogen in the blood and the more or less complete retention of the phenolsulphonephthalein also revealed a condition of severe nephritis and renal insufficiency.

The transitory character of uranium nephritis is illustrated by the last three experiments. After the sixth or seventh day the phenolsulphonephthalein excretion begins to improve. The improvement, however, may not be quite so rapid as is indicated by the percentage of the phenolsulphonephthalein excretion recorded in the tables, because relatively large amounts of the chemical may have accumulated in the animals as a result of the repeated injections during several preceding days when practically none of it was eliminated. The retention nitrogen in the blood, therefore, remains very high, even after there has been an apparently marked improvement in the phenolsulphonephthalein excretion. By the eleventh day the kidney appears practically normal again, but the non-protein nitrogen and urea are still slightly increased. The urine is free from albumin and casts, and the phenolsulphonephthalein excretion has returned practically to normal. It is evident from this series of animals that these tests of blood and urine varied with the intensity of the nephritis and in general paralleled each other.

In order to compare more accurately the relation of the accumulation of the non-protein nitrogenous products in the blood with the excretion of phenolsulphonephthalein at different stages of acute nephritis, a second series of experiments was made. In this series the blood was collected every two or three days from the ear veins and on the same days the phenolsulphonephthalein test was applied. Experiments 10 to 19, recorded below, give the salient points of these observations.



*Experiment 10.*—Rabbit 669. Urine normal; blood normal. Animal found dead on the fifth day after the administration of the uranium nitrate.

First day (wt. 1,850 gm.): Phenolsulphonephthalein excretion....75 per cent.

Blood: non-protein nitrogen (per 100 gm.).....29 mg.

Administered 3 mg. uranium nitrate.

Third day (wt. 1,830 gm.): Phenolsulphonephthalein excretion.....trace

Urine: albumin trace; epithelial cells; no casts.

Blood: non-protein nitrogen (per 100 gm.).....49 mg.

Blood: urea nitrogen (per 100 gm.).....30 mg.

Fifth day: Phenolsulphonephthalein excretion.....anuric

Blood: non-protein nitrogen (per 100 gm.).....120 mg.

Blood: urea nitrogen (per 100 gm.).....82 mg.

*Experiment 11.*—Rabbit 671. Urine normal. High initial nitrogen in the blood. Found dead on the seventh day after the administration of the uranium.

First day (wt. 1,580 gm.): Phenolsulphonephthalein excretion....80 per cent.

Blood: non-protein nitrogen (per 100 gm.).....45 mg.

Blood: urea nitrogen (per 100 gm.).....26 mg.

Administered 1.25 mg. uranium nitrate.

Fourth day (wt. 1,520 gm.): Phenolsulphonephthalein excretion.....trace

Blood: non-protein nitrogen (per 100 gm.).....80 mg.

Blood: urea nitrogen (per 100 gm.).....55 mg.

Urine: albumin large trace; a few casts; epithelial cells.

Seventh day (wt. 1,400 gm.): Phenolsulphonephthalein excretion.....trace

Blood: non-protein nitrogen (per 100 gm.).....250 mg.

Blood: urea-nitrogen (per 100 gm.).....201 mg.

Urine: albumin trace; sediment not done.

*Experiment 12.*—Rabbit 670. Urine normal; high initial nitrogen in blood. Found dead on the ninth day after the administration of the uranium.

First day (wt. 1,270 gm.): Phenolsulphonephthalein excretion....71 per cent.

Blood: non-protein nitrogen (per 100 gm.).....45 mg.

Blood: urea nitrogen (per 100 gm.).....25 mg.

Administered 1.25 mg. uranium nitrate.

Fourth day (wt. 1,250 gm.): Phenolsulphonephthalein excretion.....trace

Blood: non-protein nitrogen (per 100 gm.).....100 mg.

Blood: urea nitrogen (per 100 gm.).....74 mg.

Urine: albumin trace; a few casts; epithelium.

Seventh day (wt. 1,060 gm.): Phenolsulphonephthalein excretion....12 per cent.

Blood: non-protein nitrogen (per 100 gm.).....190 mg.

Blood: urea nitrogen (per 100 gm.).....161 mg.

Urine: many casts; no albumin.

Ninth day (wt. 950 gm.): Phenolsulphonephthalein excretion....43 per cent.

Blood: non-protein nitrogen (per 100 gm.).....95 mg.

Blood: urea nitrogen (per 100 gm.).....60 mg.

Urine: No albumin; many casts.

*Experiment 13.*—Rabbit 535. Urine normal, high initial nitrogen in the blood. Killed on the thirteenth day after the administration of uranium.

First day (wt. 1,620 gm.): Phenolsulphonephthalein excretion....70 per cent.

Blood: non-protein nitrogen (per 100 gm.).....50 mg.

Blood: urea nitrogen (per 100 gm.).....30 mg.

Administered 1.25 mg. uranium nitrate.

Fourth day (wt. 1,630 gm.): Phenolsulphonephthalein excretion....51 per cent

Blood: non-protein nitrogen (per 100 gm.).....50 mg.

Blood: urea nitrogen (per 100 gm.).....23 mg.

Urine: albumin trace; epithelial cells and casts absent.

Seventh day (wt. 1,630 gm.): Phenolsulphonephthalein excretion...48 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....50 mg.  
 Blood: urea nitrogen (per 100 gm.).....25 mg.  
 Urine: albumin large trace; sediment negative.

Ninth day (wt. 1,600 gm.): Phenolsulphonephthalein excretion...62 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....30 mg.  
 Blood: urea nitrogen (per 100 gm.).....16 mg.  
 Urine: albumin negative; a few casts.

Eleventh day (wt. 1,660 gm.): Phenolsulphonephthalein excretion...70 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....30 mg.  
 Blood: urea nitrogen (per 100 gm.).....16 mg.  
 Urine: no albumin; rare cast.

Fourteenth day (wt. 1,660 gm.): Phenolsulphonephthalein excretion, 69 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....32 mg.  
 Blood: urea nitrogen (per 100 gm.).....20 mg.  
 Urine: normal.

Histological Findings: Glomeruli negative. Tubules negative; no excess of fat; no cellular infiltration.

*Experiment 14.*—Rabbit 534. Pregnant. Urine and blood normal. Killed on the thirteenth day after the administration of uranium.

First day (wt. 2,220 gm.): Phenolsulphonephthalein excretion.....70 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....30 mg.  
 Administered 3 mg. uranium nitrate.

Third day (wt. 2,210 gm.): Phenolsulphonephthalein excretion.....trace  
 Blood: non-protein nitrogen (per 100 gm.).....45 mg.  
 Blood: urea nitrogen (per 100 gm.).....30 mg.  
 Urine: albumin large trace; epithelial cells; no casts.

Fifth day: Phenolsulphonephthalein excretion.....trace  
 Blood: non-protein nitrogen (per 100 gm.).....112 mg.  
 Blood: urea nitrogen (per 100 gm.).....82 mg.  
 Urine: albumin trace; epithelium; many casts.

Seventh day (wt. 2,100 gm.): Phenolsulphonephthalein excretion...25 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....102 mg.  
 Blood: urea nitrogen (per 100 gm.).....77 mg.  
 Urine: albumin trace; many casts.

Ninth day (wt. 1,190 gm.): Phenolsulphonephthalein excretion....40 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....59 mg.  
 Blood: urea nitrogen (per 100 gm.).....40 mg.  
 Urine not examined.

Tenth day: Phenolsulphonephthalein excretion.....46 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....45 mg.  
 Blood: urea nitrogen (per 100 gm.).....25 mg.  
 Urine: albumin trace; many casts.

Twelfth day (wt. 1,800 gm.): Phenolsulphonephthalein excretion...31 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....35 mg.  
 Blood: urea nitrogen (per 100 gm.).....23 mg.  
 Urine not examined.

Fourteenth day: Phenolsulphonephthalein excretion.....36 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....57 mg.  
 Blood: urea nitrogen (per 100 gm.).....30 mg.  
 Urine: albumin trace; no casts.

Histological Findings: Glomeruli negative. A few tubules showed regeneration and a few showed casts. Slight amount of fat in some tubules. No cellular infiltration.

*Experiment 15.*—Rabbit 536. Urine normal; blood normal. Killed on the eighteenth day after the administration of uranium.

First day (wt. 1,620 gm.): Phenolsulphonephthalein excretion.....78 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....31 mg.  
 Blood: urea nitrogen (per 100 gm.).....15 mg.  
 Administered 1.25 mg. uranium nitrate.

Fourth day (wt. 1,570 gm.): Phenolsulphonephthalein excretion.....trace  
 Blood: non-protein nitrogen (per 100 gm.).....50 mg.  
 Blood: urea nitrogen (per 100 gm.).....23 mg.  
 Urine: albumin trace; epithelium; a few casts.

Seventh day (wt. 1,530 gm.): Phenolsulphonephthalein excretion....8 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....97 mg.  
 Blood: urea nitrogen (per 100 gm.).....74 mg.  
 Urine: albumin trace; many casts.

Ninth day (wt. 1,460 gm.): Phenolsulphonephthalein excretion....52 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....47 mg.  
 Blood: urea nitrogen (per 100 gm.).....27 mg.  
 Urine: no albumin; many casts.

Eleventh day (wt. 1,630 gm.): Phenolsulphonephthalein excretion..58 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....40 mg.  
 Blood: urea nitrogen (per 100 gm.).....25 mg.  
 Urine: albumin trace; a few casts.

Fourteenth day (wt. 1,540 gm.): Phenolsulphonephthalein excretion, 65 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....32 mg.  
 Blood: urea nitrogen (per 100 gm.).....18 mg.  
 Urine: albumin negative; a few casts.

Sixteenth day: Phenolsulphonephthalein excretion.....62 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....32 mg.  
 Blood: urea nitrogen (per 100 gm.).....16 mg.  
 Urine: albumin negative; a few casts.

Nineteenth day (wt. 1,580 gm.): Phenolsulphonephthalein excretion, 72 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....26 mg.  
 Blood: urea nitrogen (per 100 gm.).....13 mg.  
 Urine: normal.

Histological Findings: Glomeruli negative. Tubules negative; no excess of fat; no cellular infiltration.

*Experiment 16.*—Rabbit 681. Urine: albumin a trace; no casts. Blood normal.

First day (wt. 2,130 gm.): Phenolsulphonephthalein excretion.....62 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....32 mg.  
 Blood: urea nitrogen (per 100 gm.).....21 mg.  
 Administered 3 mg. uranium nitrate.

Third day (wt. 2,080 gm.): Phenolsulphonephthalein excretion....14 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....47 mg.  
 Blood: urea nitrogen (per 100 gm.).....25 mg.  
 Urine: albumin trace; epithelial cells; no casts.

Fifth day (wt. 1,940 gm.): Phenolsulphonephthalein excretion.....trace  
 Blood: non-protein nitrogen (per 100 gm.).....85 mg.  
 Blood: urea nitrogen (per 100 gm.).....60 mg.  
 Urine: albumin trace; epithelial cells; no casts.

Seventh day (wt. 1,930 gm.): Phenolsulphonephthalein excretion...23 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....90 mg.  
 Blood: urea nitrogen (per 100 gm.).....68 mg.  
 Urine: albumin trace; rare cast.

- Eighth day (wt. 1,900 gm.): Phenolsulphonephthalein excretion....40 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....80 mg.  
 Blood: urea nitrogen (per 100 gm.).....56 mg.  
 Urine: albumin trace; casts.
- Tenth day (wt. 1,770 gm.): Phenolsulphonephthalein excretion....58 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....54 mg.  
 Blood: urea nitrogen (per 100 gm.).....40 mg.  
 Urine: albumin slight trace; many casts.
- Twelfth day (wt. 1,800 gm.): Phenolsulphonephthalein excretion....64 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....36 mg.  
 Blood: urea nitrogen (per 100 gm.).....19 mg.  
 Urine: no albumin; many casts.
- Fourteenth day (wt. 1,750 gm.): Phenolsulphonephthalein excretion, 48 pct. (?)  
 Blood: non-protein nitrogen (per 100 gm.).....27 mg.  
 Blood: urea nitrogen (per 100 gm.).....18 mg.  
 Urine: no albumin; few casts.
- Sixteenth day: Phenolsulphonephthalein excretion.....68 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....28 mg.  
 Blood: urea nitrogen (per 100 gm.).....19 mg.  
 Urine: no albumin; few casts.
- Experiment 17.*—Rabbit 679. Urine normal; blood normal.
- First day (wt. 2,620 gm.): Phenolsulphonephthalein excretion.....71 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....28 mg.  
 Blood: urea nitrogen (per 100 gm.).....18 mg.  
 Administered 3 mg. uranium nitrate.
- Third day (wt. 2,630 gm.): Phenolsulphonephthalein excretion....52 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....28 mg.  
 Blood: urea nitrogen (per 100 gm.).....18 mg.  
 Urine: albumin trace; epithelium; no casts.
- Fifth day (wt. 2,470 gm.): Phenolsulphonephthalein excretion....14 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....52 mg.  
 Blood: urea nitrogen (per 100 gm.).....31 mg.  
 Urine: albumin trace; a few casts.
- Seventh day (wt. 2,570 gm.): Phenolsulphonephthalein excretion...18 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....55 mg.  
 Blood: urea nitrogen (per 100 gm.).....32 mg.  
 Urine: albumin large trace; a few casts.
- Eighth day (wt. 2,500 gm.): Phenolsulphonephthalein excretion....37 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....55 mg.  
 Blood: urea nitrogen (per 100 gm.).....32 mg.  
 Urine: albumin trace; casts.
- Tenth day (wt. 2,600 gm.): Phenolsulphonephthalein excretion....69 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....50 mg.  
 Blood: urea nitrogen (per 100 gm.).....37 mg.  
 Urine: no albumin; few casts.
- Twelfth day (wt. 2,500 gm.): Phenolsulphonephthalein excretion....56 pct. (?)  
 Blood: non-protein nitrogen (per 100 gm.).....35 mg.  
 Blood: urea nitrogen (per 100 gm.).....19 mg.  
 Urine: albumin negative; a few casts.
- Fourteenth day (wt. 2,575 gm.): Phenolsulphonephthalein excretion, 75 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....27 mg.  
 Blood: urea nitrogen (per 100 gm.).....18 mg.  
 Urine normal.



*Experiment 18.*—Rabbit 680. Urine normal; blood normal.

- First day (wt. 2,180 gm.): Phenolsulphonephthalein excretion....68 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....29 mg.  
 Blood: urea nitrogen (per 100 gm.).....18 mg.  
 Administered 3 mg. uranium nitrate.
- Third day (wt. 2,150 gm.): Phenolsulphonephthalein excretion....60 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....35 mg.  
 Blood: urea nitrogen (per 100 gm.).....20 mg.  
 Urine: albumin trace; no epithelium; no casts.
- Fifth day (wt. 2,030 gm.): Phenolsulphonephthalein excretion....49 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....35 mg.  
 Blood: urea nitrogen (per 100 gm.).....19 mg.  
 Urine: albumin trace; epithelial cells present.
- Seventh day (wt. 2,070 gm.): Phenolsulphonephthalein excretion...62 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....35 mg.  
 Blood: urea nitrogen (per 100 gm.).....20 mg.  
 Urine: albumin trace; casts.
- Eighth day (wt. 2,000 gm.): Phenolsulphonephthalein excretion...64 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....32 mg.  
 Blood: urea nitrogen (per 100 gm.).....19 mg.  
 Urine: albumin negative; casts.
- Tenth day (wt. 2,020 gm.): Phenolsulphonephthalein excretion....67 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....26 mg.  
 Blood: urea nitrogen (per 100 gm.).....13 mg.  
 Urine: no albumin; few casts.

*Experiment 19.*—Rabbit 682. Urine normal; blood normal.

- First day (wt. 2,050 gm.): Phenolsulphonephthalein excretion...60 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....20 mg.  
 Blood: urea nitrogen (per 100 gm.).....18 mg.  
 Administered 3 mg. uranium nitrate.
- Third day (wt. 2,030 gm.): Phenolsulphonephthalein excretion....25 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....29 mg.  
 Blood: urea nitrogen (per 100 gm.).....18 mg.  
 Urine: albumin trace; epithelium; no casts.
- Fifth day (wt. 1,930 gm.): Phenolsulphonephthalein excretion.....trace  
 Blood: non-protein nitrogen (per 100 gm.).....55 mg.  
 Blood: urea nitrogen (per 100 gm.).....36 mg.  
 Urine: albumin trace; few casts.
- Seventh day (wt. 1,935 gm.): Phenolsulphonephthalein excretion...18 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....62 mg.  
 Blood: urea nitrogen (per 100 gm.).....40 mg.  
 Urine: albumin trace; few casts.
- Eighth day (wt. 1,870 gm.): Phenolsulphonephthalein excretion...38 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....72 mg.  
 Blood: urea nitrogen (per 100 gm.).....52 mg.  
 Urine: albumin trace; casts.
- Tenth day (wt. 2,020 gm.): Phenolsulphonephthalein excretion....55 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....47 mg.  
 Blood: urea nitrogen (per 100 gm.).....25 mg.  
 Urine: albumin negative; casts.
- Twelfth day (wt. 1,970 gm.): Phenolsulphonephthalein excretion...62 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....37 mg.  
 Blood: urea nitrogen (per 100 gm.).....20 mg.  
 Urine: albumin trace; few casts.
- Fourteenth day (wt. 1,950 gm.): Phenolsulphonephthalein excretion, 60 per cent.  
 Blood: non-protein nitrogen (per 100 gm.).....30 mg.  
 Blood: urea nitrogen (per 100 gm.).....18 mg.  
 Urine: albumin negative; few casts.

Three of the ten rabbits used in this series (Experiments 11, 12 and 13) showed a retention of nitrogen of 45, 45 and 50 milligrams, respectively, per 100 grams of blood, as against the normal figure of about 30 milligrams. This corresponds with what Folin and Denis have found in man, where the strictly normal is under 30 milligrams, yet where numerous exceptions are found among hospital patients. Two of these three rabbits died during the experiment, though they had been given only 1.25 milligrams uranium nitrate.

The correspondence between the results of the phenolsulphonephthalein tests and those of the blood analyses in Experiments 10 to 19 is quite remarkable, and in this series the significance of the former cannot have been obscured by accumulations of the drug in the body.

The correspondence is shown graphically in the accompanying charts, (a) with reference to a moderate degree of nephritis (Exp. 17), (b) with reference to severe nephritis (Exp. 16).

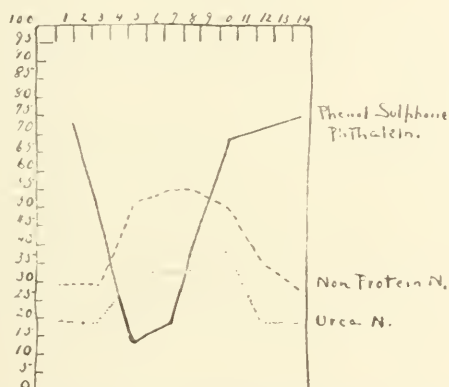


Chart 1.—Curves showing phenolsulphonephthalein excretion, non-protein nitrogen and urea nitrogen in a moderate nephritis, Experiment 17.

From the tables and the charts it is clear that at the beginning of the nephritis the phenolsulphonephthalein elimination drops more rapidly than the accumulation of non-protein nitrogen and the urea of the blood. During the course of the disease the height of the nitrogenous accumulation is reached from two to three days later than the lowest level of the phenolsulphonephthalein excretion. And after the kidney function has begun to improve, as shown by an increasing elimination of phenolsulphonephthalein in the urine, the retention nitrogen does not at once begin to recede, but may even continue to rise for a day or two, and does not reach the normal till a day or two after the phenolsulphonephthalein excretion. This is only what one must expect in view of the fact that the retention nitrogen represents the difference between that eliminated and that produced in the tissues, whereas the phenolsulphonephthalein

is an indication of the elimination alone. This is an essential difference between the two tests. The percentage of phenolsulphonephthalein excreted affords an index to the renal function at the time the test is made and the result is not visibly influenced by the length of time the kidney may have been in the condition indicated by the test. In the earliest stages of acute nephritis, such as is involved in these experiments, the nitrogen of the blood, on the other hand, does not necessarily reveal the intensity of the disease.

#### CONCLUSIONS

1. In acute uraemic nephritis in rabbits the excretion of phenolsulphonephthalein in the urine and the amount of non-protein nitrogen and urea in the blood vary from the normal during the course of the nephritis and return to normal as the nephritis heals.

2. The degree of variation from the normal agrees on the whole with the amount of destruction demonstrated histologically in the kidney.

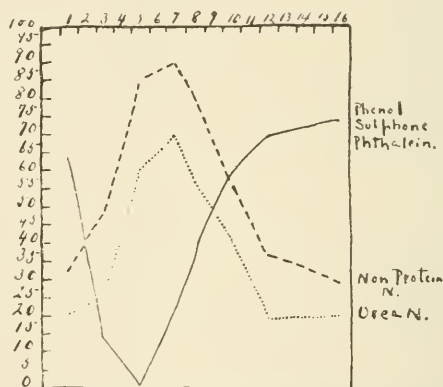


Chart 2.—Curves showing phenolsulphonephthalin excretion, non-protein nitrogen and urea nitrogen in severe nephritis, Experiment 16.

3. The phenolsulphonephthalein excretion in the urine drops rapidly to its lowest point and returns rapidly to normal with recovery of the kidney.

4. Non-protein nitrogen and urea accumulate gradually in the blood and return to normal gradually as the kidney recovers.

5. In general these tests parallel each other as indicators of renal function, but have this essential difference: the amount of phenolsulphonephthalein excretion shows the renal function at the moment; the amount of non-protein nitrogen and urea in the blood is rather a measure of an accumulating difference between the amounts of waste nitrogen produced in the metabolism and the amounts eliminated by the kidneys. The time element, the duration of the condition, is therefore an important factor in this test.

A CASE OF VEGETATIVE ENDOCARDITIS CAUSED BY A  
HITHERTO UNDESCRIBED SPIRILLUM.  
(SPIRILLUM SURATI, N. S.)\*

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During the summer of 1911, we had the opportunity of observing the course of an unusual case of vegetative endocarditis, of recovering a hitherto undescribed spiral organism from the blood in pure culture on two separate occasions, and of confirming the clinical diagnosis and recovering the same organism from the blood, cardiac vegetations and liver at autopsy.

The causal relationship of this organism to the disease in question seems definite and well established.

CASE REPORT

The patient, a man aged 37, was admitted to the Presbyterian Hospital, July 13, 1911, in the service of Dr. Bovaird, to whom we are greatly indebted for permission to use the clinical records.

*History.*—There was nothing in the family history bearing on the case. As a baby he had pleurisy and at the age of 4 suffered from an attack of diphtheria. In 1896 he was ill for three weeks with a fever of unknown cause. Ten years later he suffered from cardiac decompensation due to aortic regurgitation. So far as known he had never suffered from rheumatism in any of its forms, unless the attack of fever in 1896 was of this nature. He never had malaria and gave no history of cough or loss of weight. For seven and one-half years previous to his admission he had been a missionary in the tropics, Surat Province, Bombay, India. He always took every precaution to avoid the various diseases prevalent there. Habits good. Not alcoholic.

Onset of present trouble March 12, 1911. He had been working hard in the intense heat of the tropics when he began to have chilly sensations, moderate fever and anorexia. No malarial organisms were found in his blood and in three days he was feeling well again.

Six days later, while on the ship for America, he had another similar attack, lasting for a few days. He did not take quinin. During the remainder of the trip he continued well.

In the middle of May he had a definite chill with fever lasting several hours. The following day he felt well. Two weeks later he had a similar attack. After this he was feverish at times and had slight morning anorexia and afternoon languor.

Two weeks before admission he had a very profuse night sweat and one week later he went to bed with a temperature of 104 F. An irregular temperature persisted, being normal in the morning and about 102 F. in the afternoon. He

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\* Submitted for publication July 12, 1913.

\* From the Pathological Laboratory, Presbyterian Hospital, Columbia University, New York.



had regular night sweats but no cough or loss of weight. His sputum showed no tubercle bacilli. Appetite continued fairly good. Had no muscle or abdominal pain, no puffiness about the eyes and no urinary symptoms. Ever since going to the tropics he had had about two loose movements a day, containing a trace of fresh blood from hemorrhoids.

*Examination.*—Tall, ♂ ant and hollow chested. Not dyspneic nor cyanotic. Looked worn and tired rather than acutely ill. Eyes normal except for a little muddiness of the sclerae. Face thin and sallow. Buccal mucous membrane, pharynx and tonsils normal. Lungs clear.

The apex impulse of the heart was of heaving character in the fifth space, 11 cm. to the left of the mid-line. The left limit of dulness was 1 cm. further to the left and the right limit at the right sternal margin. At the apex there were three murmurs: a short presystolic rumble; a soft, systolic blow, transmitted to the left axilla and heard over the whole precordium; a faint diastolic blow which increased in intensity as it was traced inwards. The pulmonic second sound was accentuated and there was a loud systolic murmur in this region. Over the aortic area there was a soft systolic murmur, transmitted upwards to the clavicle. Over the upper part of the sternum and along its left border, one heard a diastolic blow, loudest at the third costal cartilage.

The pulses were equal and of distinct Corrigan quality. The radial arteries were slightly thickened.

The abdomen was soft and showed nothing abnormal. The liver was not palpable. The sharp, firm edge of the spleen descended about 1 cm. below the costal margin on deep inspiration. The extremities and reflexes were normal. No petechiae were found.

The diagnosis on admission was chronic malaria and chronic cardiac valvular disease.

July 20. On admission the patient's strength was good. A blood count showed hemoglobin, 88 per cent.; red blood-cells, 4,200,000; white cells, 10,000; polymorphonuclears, 88 per cent.; lymphocytes, 11 per cent.; eosinophils, 1 per cent. No malarial organisms found on repeated examinations at various times of the day. Quinin had no effect; von Pirquet reaction negative. In reviewing the case at this time it seemed probable that the patient was suffering from acute infectious endocarditis.

July 23. Blood culture taken July 19 is sterile. Complaints of pain in the calf of his leg. This is thought to be due to an embolus in a small artery. Two days ago the blood-count was: white cells, 15,200; polymorphonuclears, 82 per cent.; lymphocytes, 16 per cent.; eosinophils, 1 per cent.; basophils, 1 per cent. No malarial organisms found. No ova or parasites found in stool.

July 27. Fever still resists heavy doses of quinin. Physical examination remains unchanged.

August 1. Condition about the same. The irregular fever, sweats and chilliness continue. No petechiae have been found. Has two to four loose movements a day in which no ova or parasites have been found.

August 12. Has begun to lose appetite and has a little nausea. The blood count of August 3 showed: white cells, 11,500; polymorphonuclears, 77 per cent.; lymphocytes, 2 per cent.; large mononuclears, 15 per cent.; transitionals, 6 per cent. The cardiac dulness is 2 cm. further to the left.

August 17. Has been up and about for two or three days. Now has a little edema about the ankles. The blood count three days ago was: white cells, 21,000; polymorphonuclears, 90 per cent.; large mononuclears, 6 per cent.; lymphocytes, 4 per cent.; hemoglobin, 70 per cent. Wassermann reaction negative.

August 24. Grew steadily weaker and died this afternoon. Pulse continued rapid and irregular, heart dilated. Persistent abdominal distention. Labored respiration and moderate pulmonary edema. No petechiae found until the last two days when a few were seen over the thorax and one in the left conjunctiva. Two days before death the blood-count was: white cells, 36,400; polymorphonu-

clears, 88 per cent.: transitionals, 2 per cent.: lymphocytes, 9 per cent.: large mononuclears, 1 per cent.

During the course of the disease the temperature continued irregular. In the morning it would be normal or subnormal. In the afternoon the rise varied from 99.5 to 103 F. During the last two days it was continuously subnormal.

The pulse varied between 70 and 100 until near the end when it became much more rapid and irregular.

On repeated examinations the urine showed occasional hyaline casts and on one occasion a faint trace of albumin.

The patient was given four intravenous injections of collargol without any effect.

*Necropsy.*—The autopsy was performed six hours *post-mortem*. The body is that of a large framed man, showing considerable wasting. There is a little puffy edema over the dorsum of each foot. Scattered over the lower thorax and abdomen there are about a dozen small petechial spots and one is found in the left conjunctiva. The subcutaneous fat is pale and edematous.

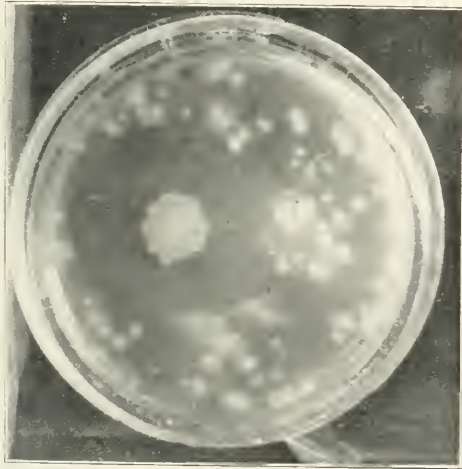


Fig. 1.—Cultures of the organism. (*Spirillum suroti*). Appearance of glucose ascitic agar plate at the end of a week. Natural size.

On opening the thorax a broad precordial surface is exposed, the lung borders being well retracted to either side. Each pleural cavity is about one-third full of clear, light, amber-colored fluid. There are no adhesions present. The left lung weighs 500 gm. The surface is smooth. There are some areas of emphysema along the anterior border. The posterior and lower portions are congested. On section the lung is bright red in color and moderately edematous. It is air-containing and shows no areas of consolidation. The vessels are clear and the bronchi normal. The right lung weighs 560 gm. It is similar in all respects to the other except that it is a little more congested. The bronchial lymph-nodes are moderately pigmented but otherwise normal.

The pericardial sac contains about 300 c.c. of clear, light amber colored fluid. The pericardial surfaces are smooth and glistening. The right side of the heart is moderately enlarged and contains post-mortem clot. The heart weighs 550 gm. The tricuspid valve admits the tips of four fingers. The cusps are delicate and show no vegetations. The right ventricular wall measures 6 to 8 mm. in thickness. It is pale brown in color and somewhat muddy in appearance. Towards the lower part of the ventricle the muscle becomes more cloudy yellow in color.

The pulmonary valve is delicate and competent. The left side of the heart is moderately enlarged. The wall of the ventricle averages about 1.5 cm. in thickness. At a point about 6 cm. below the mitral ring the heart muscle becomes dull-yellow in color in contrast to the more reddish-brown appearance above. This area joins with the one in the right ventricle. The branches of the coronary artery supplying this region are perfectly patent and there is no evidence of any thrombus. The mitral valve appears as a button-hole slit. The cusps are fused together, thickened, moderately calcareous and somewhat retracted, forming a rigid ring of tissue and causing a marked stenosis of the orifice. Along the line of closure of the cusps and extending for a short distance on their upper and under surfaces, there are numerous small, soft vegetations with a little super-

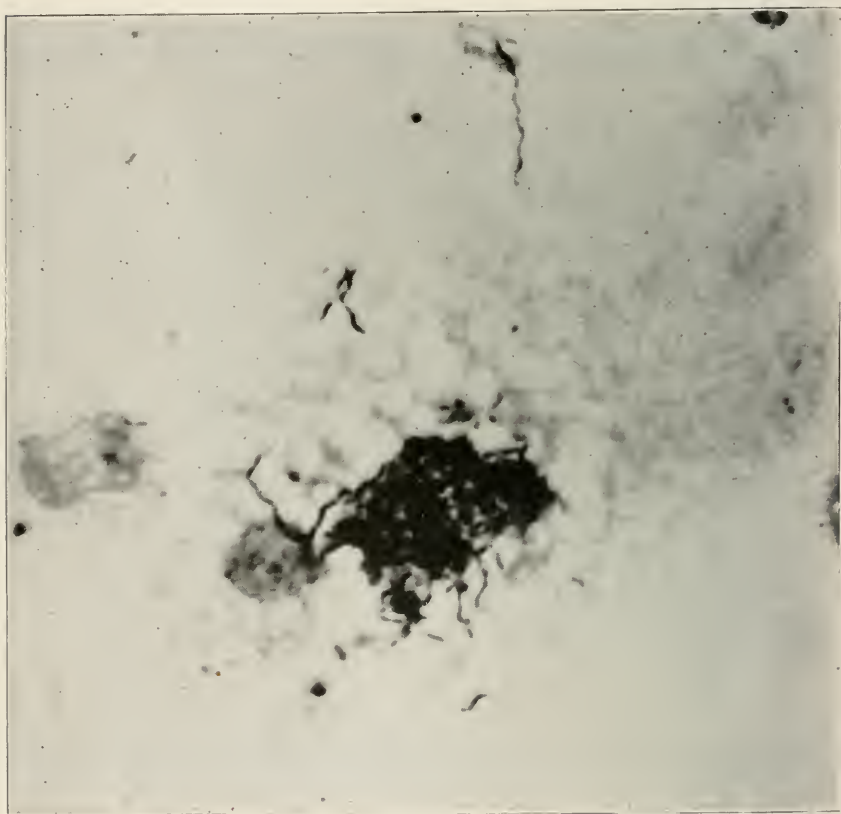


Fig. 2.—Stained preparation from the original blood-culture at the end of eight days. (Magnification 1:1900.)

ficial ulceration. On opening the aorta, the site of the aortic valve is marked by a large, irregular, cauliflower-like vegetation of soft consistence. It is about the size of an English walnut and completely fills the orifice with the exception of small openings through the vegetation itself. One of these openings is in front, one behind and the third through the middle of the mass. The cusps are much distorted and fused together. The vegetations do not involve the wall of the heart, the papillary muscles or chordae tendinae. At the base of the aorta there are a few streaks of yellowish atheroma. The rest of the aorta is elastic

and moderately smooth except in the lower portion where there are a few chalky deposits. The openings of the coronary arteries are free from sclerosis. In their course they show a little scattered atheroma, but at no point is this marked nor is their lumen obstructed in any way.

The abdomen contains about 500 c.c. of clear, amber fluid. The peritoneal surfaces are smooth and glistening. The spleen weighs 450 gm. It is not adherent to the surrounding structures. On section it is dark purplish-red in color and



Fig. 3.—Stained preparation from the original blood-culture at the end of two weeks. (Magnification 1-1900.)

the splenic pulp is very firm, the cut surface remaining perfectly flat. There are no infarcts present. The liver weighs 1,650 gm. Its surface is smooth, consistence firm and color muddy brown. On section it has the appearance of chronic passive congestion. The gall-bladder and ducts are normal. The left kidney



weighs 200 gm. Its capsule is slightly adherent. The surface is smooth and of normal color. The kidney is firm. Near the upper pole there is a small infarct. On section the kidney has the appearance of chronic passive congestion and cloudy swelling. The pelvis and ureter are normal. The right kidney weighs 170 gm. It is similar in all respects to the other except that there are no infarcts present. The adrenals appear perfectly normal. The pancreas is firm and of normal color. At its widest part it measures 6 cm. It appears normal on section. The ducts are normal. The esophagus, stomach and intestines show no change. The mesenteric lymph-nodes are slightly enlarged. The prostate and bladder are normal. The brain was not examined.



Fig. 4.—Preparation from a deep ascitic glucose agar culture forty-eight hours old. Dark field illumination. (Magnification 1-1100.)

*Microscopical Examination.*—Heart muscle: The pericardium is a little edematous and shows some infiltration with mononuclear wandering cells. The muscle fibers just beneath the pericardium and endocardium are pale, swollen and degenerated. In general, the heart muscle has the appearance of cloudy swelling. Corresponding with the dirty yellow area described on gross examination, the picture is quite different. Here one sees many polymorphonuclear leukocytes scattered diffusely between the muscle bundles and even between the individual fibers. They are seen especially about the vessels and these are congested and show many polymorphonuclear leukocytes in the lumina. Some of the very small vessels are thrombosed. Many of the muscle fibers are quite granular and show no nuclei.

**Mitral and Aortic Vegetations:** Sections show structureless, pinkish material with some fibrin, blood-platelets and polymorphonuclear leukocytes. In some areas there are small hemorrhages. Just beneath the free surfaces and extending irregularly throughout the vegetations, one sees many dense masses and scattered clumps of bacteria. With the hemotoxylin-eosin stain it is difficult to make out the form of these organisms.

**Lungs:** Show chronic passive congestion and edema.

**Spleen:** Shows nothing more than well marked chronic passive congestion.

**Liver:** The same process is very marked in the liver, fully one-half of each lobule being affected. Otherwise, the liver cells show cloudy swelling and some fatty infiltration.



Fig. 5.—From an ascitic fluid tissue culture covered with paraffin oil. Ten days old. Dark field illumination. (Magnification 1-1100.)

**Kidney:** There is a typical infarct. There is also general congestion and cloudy swelling. The glomeruli were carefully searched for any evidences of the lesions which have been described as occurring with subacute vegetative endocarditis, but no such changes were found.

**Pancreas:** Normal.

**Adrenals:** Normal.

#### SPECIMENS PREPARED WITH LEVADITI STAIN

Sections of the vegetations from the aortic and mitral valves show a very striking picture. Scattered through the sections are dense masses of bacteria stained black. In places these organisms are so densely packed together that it

is quite impossible to make out their forms. Where they are less numerous, one can see many slightly curved bacilli, comma-and S-shaped forms, thicker than in the preparations from cultures, owing to the impregnation with silver salt. Here and there in these clumps one can make out perfectly definite spirilla of varying lengths. In other portions of the sections, one finds small clumps and isolated organisms. Some of these appear as beautiful spirilla of from eight to ten turns; others as commas and S-shaped forms.

A study of these sections shows the organism appearing in its various forms as in cultures.

Sections of the liver, spleen, heart-muscle, kidney and pancreas stained by the same method fail to show any of the organisms.

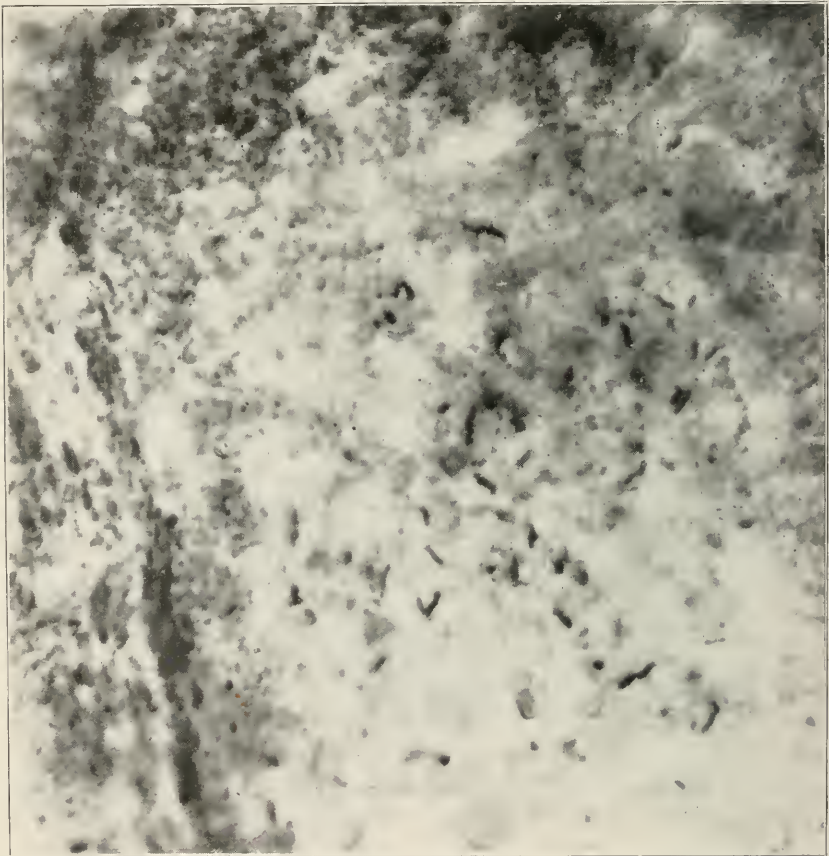


Fig. 6.—Section of aortic vegetation stained by the Levaditi method. It shows numerous small S and comma forms. (Magnification 1-1900.)

#### CULTURES TAKEN AT AUTOPSY

Cardiac Vegetations: Pure growth of the spirillum.

Heart Blood: Mixed growth of spirilla and colon bacilli.

Liver: Two cultures showed a pure growth of the spirillum and one culture gave only the colon bacillus.

Pericardial Fluid: Colon bacillus.

Pleural Fluid: Sterile.



Ascitic Fluid: Sterile.

Spleen: Sterile.

Smears: These were made from the aortic and mitral valve vegetations. They showed numerous perfectly definite spirilla of varying lengths and no other organisms.

#### BLOOD-CULTURES AND DESCRIPTION OF THE ORGANISM

A blood-culture taken July 19 remained sterile for five days, was so reported and discarded. It was the custom in the laboratory at that time to keep all blood-cultures for five days, making the final observations and reports at the end of that time.

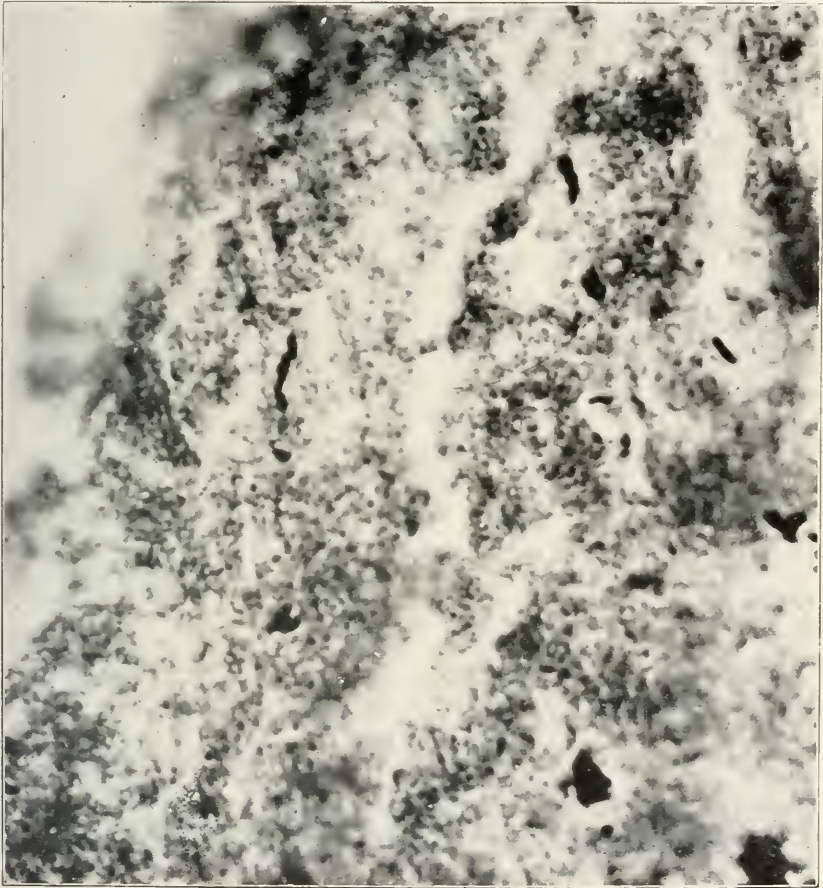


Fig. 7.—Section of aortic vegetation stained by the Levaditi method showing a short spirillum and a few of the smaller forms thickly coated with the silver salt. (Magnification 1-1900.)

The second culture was taken August 13. Different quantities of the patient's blood were added to each of three flasks of nutrient broth (250 c.c.) faintly alkaline to litmus. For four days the cultures remained sterile and were not opened. The supernatant fluid was clear and the blood and clot were unchanged. On the fifth day the cultures were examined and found sterile. The flasks were



not discarded, however. On the sixth day, the supernatant fluid in all three flasks was distinctly turbid and there was slight hemolysis present. On examining hanging drop preparations, no free organisms could be seen, but here and there were small clumps of granular material. These clumps resembled the picture seen in cultures incubated for four or five days where the red cells have begun to break up, forming little masses of debris. On the following day, the



Fig. 8.—Section of aortic vegetation stained by the Levaditi method. It shows a spirillum just to the left of the middle, rather thickly coated with silver salt. In the upper right hand corner there is an S-shaped form and in the lower left hand corner a comma form. (Magnification 1-1900.)

seventh from the time of taking the cultures, hanging drop preparations showed free spirilla making their way actively across the field. There were also many of the granular clumps similar to those seen on the previous day. From the periphery of these clumps, numerous spirilla projected and occasionally one could

be seen to break away and move very actively across the field. It was at first thought that some very unusual contamination had taken place. The two other flasks were examined and a similar picture found in each. The smaller spirilla were very actively motile, traveling across the field so rapidly and at such varying depths that it was very difficult to follow them. The longer forms were rather sluggish and stiff and showed very little flexibility of their bodies.

Drops of the broth culture stained with methylene blue or carbol-fuchsin showed the clumps already referred to. (See illustration.) These consisted of tangled masses of organisms, most of which appeared in the form of small, slightly curved bacilli, commas and S-shaped forms. Some were so small as to appear like dots. The central portions of these clumps were quite thick and one received the impression of a granular mass without being able to trace the individual forms composing it. Here and there, one could make out one of the longer spirilla and these could be well seen at the periphery. Some of the clumps were quite small, consisting of only a few spirilla and commas.

Scattered through the stained specimens were many single organisms. The smallest forms seen were slightly curved bacilli or comma forms, a little thickened at the maximum part of the curve. These averaged about 3.4 microns (0.0034 mm.) in length. Rather more common than these were the S-shaped forms which had the appearance of two of the comma forms united. From these one could trace various transitions up to long spirilla measuring as much as 23.5 microns (0.0235 mm.) in length. One could find spirilla of three or four half turns, or two S-shaped forms united by a very delicate thread. Some of the spirilla had as many as sixteen half turns. The ends of these long forms were noticeably pointed and one could make out delicate transparent areas running at right angles to the long axis. The spiral turns were not perfectly regular. Some were quite abrupt. More often they were more gradual and the organisms were slightly bent on themselves. As a rule the spirilla were quite thin and delicate, but there were many forms which were more unevenly stained giving parts of the curves a slightly thickened appearance. No spores could be demonstrated.

August 22, two days before the patient's death, a third culture was taken. All three flasks showed a pure culture of the same organism. The only difference from the previous culture was that the spirilla were recovered from the cultures one day earlier. Following the positive culture, several blood-smears were examined for spirilla with negative results.

#### SUBCULTURES

Subcultures were taken on various media and the results as given here represent the general average as found after a large number of cultures had been examined.

Plain Broth: In twenty-four hours there is a very slight, diffuse cloudiness with a slight sticky sediment at the bottom of the tube. Stained preparations show many slightly curved bacillary, comma- and S-shaped forms and a moderate number of short spirilla, not exceeding six half turns.

In forty-eight hours the cloudiness becomes more marked. The sediment at the bottom of the tube is much increased in amount and of whitish color. It is quite sticky, and on twirling the tube it forms a long, tree-like, whitish upshoot, remaining attached to the bottom of the tube when its upper end has reached the top. A little more vigorous shaking frees it and breaks it up. Stained smears now show numerous spirilla, many of which are quite long.

On longer incubation the appearance does not change appreciably, the sediment merely showing a moderate increase. Motility continues for as long as three weeks in incubated broth tubes. After this time it gradually dies out.

In broth as in the other cultures, the average time and appearance of the growth is given. When first isolated, the organism took much longer to grow and the growth was much more delicate and sparse.

**Glucose Broth:** No growth in twenty-four hours. At the end of forty-eight hours, there is a very slight cloudiness in the open arm of the fermentation tube, but none in the closed arm. No gas is formed. Smears show the short forms and a few spirilla of about four half turns. By the end of a week the broth becomes more turbid and there is a little whitish sediment on the sides of the tube which separates out into little sticky threads on shaking. No growth in the closed arm of the tube and no gas.

**Lactose Broth:** Follows the appearance of the glucose broth in every detail. No gas is formed.

**Litmus Milk:** Tubes freely inoculated with the organism never showed any change and it was never possible to demonstrate any growth in this media.

**Inulin:** Not fermented.

**Ascitic Glucose Agar:** This was the most favorable medium found. However, mediums made from different samples of ascitic fluid did not prove equally favorable. In fact, at times the organism would not grow at all on some of the samples and was finally lost in this way.

At the end of twenty-four hours there are a few minute, semi-transparent, dew-like colonies with a very slight whitish sediment in the water of condensation. Smears at this time show only the small bacillary and comma forms. In forty-eight hours the colonies are slightly larger. Older cultures show a gradual increase in the size of the colonies up to one-half or three-quarters of a cm. in diameter. They are round with a serrated or irregular border. The margins are clean cut. They are distinctly raised above the surface of the medium and are of a dirty white color. With the light shining through, the centers appear thick and brownish, the periphery white and more translucent. Under the low power of the microscope, the margins appear slightly raised. There is a suggestion of radiating lines from the periphery to the center. The sediment in the water of condensation becomes very sticky and rolled up into a little ball-like clump which can be separated out on shaking. The growth on the surface of the medium becomes quite tenacious. The superficial part can be easily removed with the platinum wire, leaving an area of the same size, translucent in appearance which cannot be removed without taking the medium with it.

**Glucose Agar:** The appearances here are the same as with the ascitic agar, but it takes the colonies about twenty-four hours longer to develop; they are never as numerous, and they do not reach the same size.

**Plain Agar:** The only difference from the above is that the growth is slower, more uncertain and never as profuse. Many times on this medium the organism fails to grow at all. \*

**Blood Agar:** In from twenty-four to forty-eight hours, minute whitish colonies appear, the red of the medium shining through. They are glistening in appearance. Smears show the same picture as in the case of the ascitic agar colonies at the same time. At the end of a week the colonies reach a size of from 3 to 4 mm. in diameter. In appearance these colonies do not differ from those on ascitic agar.

**Loeffler's Blood Serum:** The growth is generally slow and not profuse. The appearances are the same as with the other solid media.

The organism did not produce indol, did not liquefy gelatin and did not conform to the cultural characteristics of the spirillum of cholera.

**Anaerobic Cultures:** The organism is a facultative anaerobe. It grows fairly well under anaerobic conditions, appearing as a dirty white, non-transparent cloud in the media. The colonies appear somewhat granular and show a tendency to grow between the column of agar and the side of the tube. When grown under these conditions the organisms are apt to show more regular curves and to occur in longer filaments.



**Animal Inoculations:** White rats, guinea-pigs, and rabbits were inoculated intraperitoneally and intravenously with 0.5 and 1 c.c. of a twenty-four-hour broth culture without causing any symptoms. At the end of three months these animals were killed and no lesions were found. The organism could not be recovered from the blood at this time. Unfortunately these experiments could not be repeated as the organism died out and was lost.

#### STAINING

The organism stains well with the ordinary anilin dyes, but the best results are obtained with carbol-fuchsin. It is Gram-negative. Spreads made with India ink show up especially well and the organism can be well studied by means of the dark field illumination.

#### SUMMARY

We are fully aware that the report of any new organism is always open to the criticism of being a contamination or some secondary infection, having no causal relationship to the disease in question. In spite of this, however, we believe that it is of great importance to report any such organisms where there is apparently just ground for considering them pathogenic. It calls attention to new possibilities and places on record a description of the organism for future reference and comparison.

In the present case, the organism in question seems to be fairly well established as the cause of the disease. The blood-culture results were very satisfactory. On two separate occasions, with about ten days intervening, three broth flasks showed a pure growth of the organism. A third culture, the first one taken, was discarded as sterile on the fifth day. This was two days before the second culture showed a growth and one day before the last culture was positive. It is possible that had it been kept, it, too, would have been positive.

At autopsy, the same organism was obtained from the blood, liver and cardiac vegetations, several times in pure culture, in the other instances in association with the usual autopsy organism, the colon bacillus.

Smears of the vegetations showed the organism very definitely. No other variety of bacteria was seen in these smears. Sections of the vegetations also showed the organism very well and again no other bacteria could be identified.

The animal inoculations were not so satisfactory. They were carried out on only a limited scale and gave only negative results. As mentioned above, these experiments could not be repeated owing to the loss of the organism.

One of the striking things about the case was the fact that the blood-cultures did not become positive until after the time when they are usually discarded. It would seem advisable, in the light of this experience, to keep all blood cultures from unusual cases or those running a temperature for more than five days.



We have been unable to find any reference to an organism similar to the one found in this case and have ventured to give it the name *Spirillum surati*, after the name of the locality in which the disease was presumably contracted.

In conclusion, we wish to express our deep appreciation to Dr. Noguchi for his valuable assistance in the study of the organism and the preparation of this report.

## A CASE OF SPIRILLUM INFECTION \*

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The following case, while lacking autopsy confirmation, is of considerable interest when considered in conjunction with the case reported in this issue by Drs. Lamb and Paton. Like this, it suggests a form of infection probably overlooked not very infrequently.

*Case History*.—Male, aged 43, American, widower; occupation, designer. Admitted to Presbyterian Hospital September 23, 1912.

*Family History*.—No bearing.

*Past History*.—Always notably well except for typhoid fever in 1895, and two attacks of malaria in 1911 and July, 1912, respectively, parasites having been found.

*Habits*.—Moderate user of alcohol, heavy user of tobacco. No genito-urinary history obtained.

*Present Illness*.—Onset sudden on September 2, twenty-one days before admission, with severe nausea, vomiting and inability to retain any food or water. These symptoms continued for five days, at the end of which time the stomach was again apparently normal. On September 20, three days before admission, the same symptoms recurred, this time with moderate jaundice on the second day. Stools were of light color in the beginning, but soon became of normal color. Urine became and continued dark. There was some tenderness under the right costal margin, but no pain radiating from this region. There was, however, sharp pain about the left scapular spine radiating to the left inner arm, also a distinct feeling of oppression about the upper chest.

On admission the physical examination showed a well-developed middle-aged man, quite prostrated, without dyspnea. He was slightly jaundiced. The teeth were in excellent condition, tonsils not enlarged, neck not stiff. The lungs showed a few subcrepitant râles at the right base posteriorly; otherwise they are described as negative. The heart apex impulse was neither seen nor felt, no sounds were to be heard at any point, no adventitious sounds were heard. The area of cardiac dulness was greatly enlarged, extending on the right in the fourth space to 7 cm. to the right of the mid-line, in the fifth space 8 cm. to the right of the mid-line. The left limit of dulness in the third space was 10 cm. to the left of the mid-line; in the fourth space 11.5 cm. to the left of the mid-line; in the fifth space 12 cm. to the left of the mid-line. The pulses were equal and small, their tension seemed low, there was intermission about each fifth to sixth beat. The liver dulness extended up to the fourth space, with flatness from the sixth rib to the costal margin. Below the right costal margin there was a globular mass, slightly tender on palpation, dull, almost flat on percussion. The spleen was not felt. The abdomen was otherwise negative. The extremities were normal.

A tentative diagnosis was made of cholecystitis and pericarditis with effusion. A radiograph taken on the following day revealed the following condition, the report being given verbatim:

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\*From the Pathological Laboratory, Presbyterian Hospital, Columbia University, New York.

"Examination not very satisfactory on account of condition of patient; no calculi made out in gall-bladder; heart is very large and dilated; further than this, examination not satisfactory."

*Clinical Course.*—September 27 there were signs of fluid at the right base posteriorly; the chest was aspirated and about 35 ounces of clear, amber fluid was obtained. An examination of this fluid was as follows:

Quantity, 770 c.c. Specific gravity, 1.011. Sediment, bloody. Differential of 100 cells: Polymorphonuclears, 8 per cent.; lymphocytes, 42 per cent.; endothelial cells, 50 per cent. Sodium chlorid .572, which equals 4.4 gram total. Reaction for bile, positive. No bacteria found in sediment by centrifuge.

September 28 a needle was inserted into the pericardial sac in the fifth space, 11 cm. to the left of the mid-line. A bloody, gelatinous fluid was obtained, showing under the microscope many blood and pus cells and a fair number of spirilla; no other bacteria. This fluid was sterile aerobically on both broth and Loeffler's blood-serum.

At 9 p. m. on the same day, thoracotomy was done and the pericardium drained; 30 c.c. of bloody pus flowed from the pericardial sac. Examination was as follows:

Polymorphonuclears, 96 per cent.; large mononuclears, 1 per cent.; lymphocytes, 1 per cent.; endothelial cells, 2 per cent. Sediment about 7 per cent., consisting roughly of one-third pus and two-thirds blood-cells. Three clear cut spirilla found. No other bacteria. Cultures on glucose agar, ascitic glucose agar and broth aerobically sterile.

September 29, left chest aspirated; 26 ounces of bloody fluid obtained. Smears showed a few spirilla identical with those previously found; no other bacteria; no tubercle bacilli. Cultures, aerobic broth sterile; glucose agar sterile.

October 3, left chest again aspirated and 5 ounces obtained identical with that of September 29.

October 7, tender swelling of the right parotid gland. October 8, the right gland subsiding; the left parotid now somewhat swollen. From this point on the patient weakened steadily until death intervened on October 11.

Five blood-counts made during his stay showed as follows:

On admission:

White blood-cells, 15,000; polymorphonuclears, 73 per cent.

September 30:

White blood-cells, 34,000; polymorphonuclears, 91 per cent.

October 5:

White blood-cells, 15,200; polymorphonuclears, 81 per cent.

October 7:

White blood-cells, 24,400; polymorphonuclears, 84 per cent.

October 10:

White blood-cells, 41,000; polymorphonuclears, 81 per cent.

Eosinophils were never found, nor were any abnormal cells found. The temperature was on admission 103 F.; it fell gradually to normal, reaching that point on the sixth day. It then gradually rose for seven days and finally, during the last week, was of a septic type, but low—99 to 100 F.

This case was, in the beginning, considered as having its origin below the diaphragm as a process which had extended upward. It was also presumed to be due to some ordinary pyogenic organism. The assumption that the original infection was below the diaphragm was probably correct, but repeatedly sterile cultures and the absence of any organisms, save the spirilla, from the smears make the supposition of a pure

spirillum infection strong. Unfortunately, the condition of the patient was such that it seemed inadvisable to take a blood culture.

#### DESCRIPTION OF ORGANISM — MORPHOLOGY

The organism is apparently a spirillum, being made up of segments lying end to end. These segments average 0.3 to 0.5 micron in width, and 1 micron in length; their ends are rather abruptly blunt; the waves are regular, rectangular, the wave length averaging 1.2 micron.

Cultures: In the beginning these organisms were found to be anaerobic, no growth being obtained on any of the ordinary culture media aerobically. Anaerobic growth, however, was obtained on the special medium devised by Noguchi for the cultivation of *S. pallida*. After growth on this medium the organism was found to be increasingly aerobic. On this special anaerobic medium there was no coagulation of the protein material and no formation of gas. The odor was a faint musty one, otherwise not unpleasant. The subsequent aerobic growth showed on ascitic agar plates deep colonies which were of a punctate and opaque form. Superficial colonies were about 1 mm. in diameter, of regular outline and of an opaque, grayish white color. In all fluid media, in which there was to a large extent, exclusion of oxygen, by layering with paraffin oil, there was formed a deposit of faint color, in the form of a ring about the tube, between the medium and the overlying oil. As a general rule, the organism was shorter in anaerobic media. The shorter forms, which were often unicellular or composed of only two or three segments, were actively motile, both backward and forward, moving with equal facility and with an extremely rapid lateral vibration. In longer spirilla, made up from eight to twelve organisms, motility was sluggish, rather rigid, with very little flexibility. The motion was of a forward, zig-zag type, the spirilla moving as a rigid whole. The short anaerobic form, grown on the ascitic broth tissue medium, after one week became considerably longer, then appearing as a rule as a spirillum of about the same length as found in the smears of the original pus.

#### ANIMAL INOCULATIONS

The original pus and cultures were put into the following animals with no apparent effect: Rabbits, intravenously; guinea-pigs, intraperitoneally; rabbits, by injection into the testicle.

For the privilege of reporting this case I am indebted to Dr. George Tuttle. Also I desire to thank Dr. Hideyo Noguchi for very great assistance in the study of the organism.



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12. Töpfer: Wien. klin. Wehnschr., 1892, v. 49; Bondzynski and Gottlieb: Centralbl. f. d. med. Wissensch., 1897, xxxv, 577; Salkowski: Berl. klin. Wehnschr., 1910, xlvii, 1746; Hess and Saxl: Beitr. z. Carcinomforsch., 1910, Part 2; Salkowski and Kojo: Berl. klin. Wehnschr., 1910, xlvii, 2297; Kojo: Ztschr. f. physiol. Chem., 1911, lxxiii, 416; Einhorn, Kahn and Rosenbloom: Am. Jour. Gastro-Enter., 1911, i, 2; Arch. f. Verdauungskr., 1911, xvii, 557; Kahn and Rosenbloom: Biochem. Bull., 1912, ii, 87. Dr. Rosenbloom is studying the relation between "acidosis" and the amount of colloidal nitrogen in the urine.

neutral sulphur of the urine is also increased in cancer. Weiss<sup>13</sup> in a thorough study has found a high increase in the amount of neutral sulphur excreted by patients with carcinoma. His paper also contains a list of all of the neutral sulphur estimations that have been made on urine from patients suffering from various diseases. Salomon and Saxl<sup>14</sup> claim that the urine of carcinomatous individuals contains a neutral sulphur constituent, the sulphur of which can be split off by means of hydrogen peroxid. They found that urine containing this substance yielded 0.01-0.018 grams of BaSO<sub>4</sub> from this sulphur per 100 c.c. of urine. Murachi<sup>15</sup> also found this type of sulphur substances present in the urine of cancer patients. He claims it may compose as high as 3.8 per cent. of the total sulphur of the urine and that it belongs to the neutral sulphur fraction. Petersen,<sup>16</sup> in studying this reaction, found it to be positive in case of cancer. Weiss,<sup>13</sup> using the Folin methods of estimation of the sulphur partition of the urine, found in normal men that the neutral sulphur was from 12 to 40 per cent. of the total sulphur, the average being 16.5 per cent. He thinks a part of the neutral sulphur has an exogenous origin, but the greater part is endogenous, and that any condition causing an increased destruction of body protein causes an increased excretion of the neutral sulphur of the urine. He found that there was a marked increase in the neutral sulphur of urine from patients suffering from tuberculosis, while in carcinoma the neutral sulphur was from 20.3 to 36.4 per cent. of the total sulphur. Guitaro<sup>17</sup> found that in normal adult men the neutral sulphur of the urine was 19.41 per cent. of the total sulphur and in normal adult women it was 21.14 per cent. of the total sulphur.

Our work was carried out with the hope that we might be able to find that the increased neutral sulphur of the urine from patients suffering from cancer, would prove a reliable aid in the diagnosis of cancer.

## II. METHODS

The patients received the ordinary hospital diet, unless otherwise stated. The urine was collected in twenty-four-hour periods, using powdered thymol as a preservative. The total sulphur of the urine was estimated by Benedict's<sup>18</sup> method, the total and inorganic sulphates by Folin's method.<sup>19</sup> The ethereal sulphates were computed by subtracting the inorganic sulphates from the total sulphates, and the neutral sulphur

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13. Weiss: *Biochem. Ztschr.*, 1910, xxvii, 175.

14. Saxl: *Deutsch. med. Wehnschr.*, 1912, xxxviii, 53.

15. Murachi: *Biochem. Ztschr.*, 1912, xli, 138.

16. Petersen: *Deutsch. med. Wehnschr.*, 1912, xxxviii, 1536.

17. Guitaro: *Riv. crit. clin. med.*, 1910, x, 593.

18. *Jour. Biol. Chem.*, 1909, vi, 363.

19. *Jour. Biol. Chem.*, 1905-06, i, 13.

was computed by subtracting the total sulphate-sulphur from the total sulphur. The accompanying tables contain the data obtained in this study.

#### DISCUSSION OF TABLE 1

In the nine cases of diabetes studied the neutral sulphur was increased with the exception of Case 4. This case was a mild type, the urine being sugar free when the patient was on a carbohydrate free diet. The other cases were of the severe type with the exception of Case 7. It is interesting to note that the colloidal nitrogen of the urine has been found to be increased also in cases of diabetes.<sup>20</sup> In this series of estimations the average daily total sulphur excretion is 0.94 gm., the average neutral sulphur is 0.21 gm., or 22.3 per cent. of the total sulphur.

#### DISCUSSION OF TABLE 2

It may be noted that in the thirteen cases of cancer the neutral sulphur of the urine is consistently high both in amount and in proportion to the total sulphur of the urine, with the exception of Case 18, in which the neutral sulphur is not increased.<sup>21</sup> In these cases of carcinoma the lowest neutral sulphur excretion is 0.05 gm., and the highest 0.76 gm. In relation to the proportion of total sulphur excreted as neutral sulphur, the lowest is 6.2 per cent. and the highest 67.9 per cent. While the average total sulphur excreted is 0.88 gm., the average neutral sulphur is 0.20 gm. or 23.1 per cent. of the total sulphur.

#### DISCUSSION OF TABLE 3

A marked increase in the neutral sulphur of the urine was found in one case of interstitial nephritis, in one case of lobar pneumonia and in one case of hypopituitarism. In one case of interstitial nephritis and in one case of pneumonia it was not increased. Very low neutral sulphur excretions were found in two cases of chronic lead poisoning. In two cases of chronic appendicitis there was no increase. In one case of bronchial asthma there was a slight increase. In one case of hepatic abscess and in one case of syphilitic hepatitis there was no increase. In one case of perihepatitis and in one case of cholelithiasis there was an increase of the neutral sulphur of the urine, only when bile was excreted in the urine. In one case of typhoid fever, of chronic myocarditis with broken compensation, and one case of gastropotosis and gastric dilatation, there was no increased neutral sulphur excretion.

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20. Einhorn, Kahn and Rosenbloom: *Am. Jour. Gastro-Enter.*, 1911, i, 2; *Archiv. f. Verdauungskr.*, 1911, xvii, 557.

21. It is to be remembered that this case clinically resembled carcinoma of the stomach. It is possible, however, that the diagnosis was wrong. Unfortunately we were not able to follow the case to operation.

TABLE 1.—THE URINARY SULPHUR PARTITION IN DIABETES MELLITUS

279.

Case No.	Date.	Total Sulphur, gm.	Sulphate Sulphur, gm.	Ethereal Sulphate Sulphur, gm.	Inorganic Sulphate Sulphur, gm.	Neutral Sulphur, gm.	Sulphate Sulphur $\div$ Total Sulphur, Pct.	Ethereal Sulphate C. $\div$ Total Sulphur, Pct.	Inorg. Sulphate S. $\div$ Total Sulphur, Pct.	Neutral Sulphur $\div$ Total Sulphur, Pct.	Diagnosis and Remarks.
1	6/27	1.04	0.89	0.059	0.829	0.151	85.5	5.6	79.9	14.5	Diabetes mellitus with gangrene of toe; patient on strict antidiabetic diet. Urine still contains sugar.
1	7/6	1.52	0.98	0.088	0.894	0.538	64.8	5.8	59.0	35.4	
1	7/7	1.16	0.83	0.079	0.749	0.332	71.4	6.8	64.6	28.6	
1	7/8	0.91	0.77	0.087	0.684	0.139	84.7	9.5	75.2	15.2	
1	7/9	1.20	1.07	0.045	1.025	0.130	89.1	3.7	85.4	10.8	
1	7/10	1.98	1.85	0.151	1.699	0.130	93.4	7.6	85.8	6.5	
1	7/11	1.31	1.15	0.027	1.123	0.160	87.7	2.1	85.6	12.2	
1	7/12	1.30	1.15	0.089	1.061	0.150	88.4	6.8	81.6	11.5	
1	7/13	1.01	0.86	0.087	0.773	0.150	85.1	8.6	76.5	14.8	
2	7/8	0.893	0.635	0.055	0.580	0.258	71.1	6.2	64.9	28.8	Diabetes mellitus. Strict anti-diabetic diet. Urine still contains sugar.
2	7/9	0.871	0.519	0.028	0.491	0.352	59.6	3.2	56.4	40.4	
2	7/10	0.583	0.470	0.016	0.454	0.093	86.2	2.8	83.4	15.9	
2	7/12	1.320	0.963	0.014	0.949	0.357	72.9	1.1	71.8	27.1	
2	7/13	0.737	0.604	0.014	0.590	0.133	81.9	1.9	80.0	18.1	
2	7/14	0.589	0.475	0.014	0.461	0.114	80.8	2.4	78.4	19.4	
2	7/15	0.750	0.623	0.013	0.610	0.127	83.1	1.7	81.4	16.9	
3	6/27	1.61	1.216	0.073	1.143	0.394	75.5	4.53	70.97	24.4	Diabetes mellitus with gangrene of foot. Strict anti-diabetic diet. Urine still contains sugar.
3	6/28	0.872	0.626	0.092	0.614	0.246	71.8	10.6	61.2	28.2	
3	7/6	1.686	1.20	0.091	1.109	0.486	71.1	5.4	65.7	28.8	
3	7/7	0.369	0.226	0.027	0.199	0.143	61.3	7.3	54.0	38.8	
3	7/8	0.454	0.241	0.051	0.190	0.213	53.1	11.2	41.9	46.9	
3	7/9	1.12	0.865	0.013	0.852	0.255	77.2	1.16	76.04	22.8	
3	7/10	1.20	0.989	0.098	0.891	0.211	82.4	8.1	74.3	17.5	
3	7/11	0.554	0.287	0.002	0.285	0.267	51.8	3.6	48.2	48.2	Diabetes mellitus with gangrene of foot. Strict anti-diabetic diet. Urine is free from sugar.
3	7/12	1.404	1.09	0.061	1.029	0.314	77.6	4.3	73.3	22.4	
4	7/6	0.84	0.79	0.03	0.76	0.05	94.0	3.6	90.4	5.9	
4	7/8	1.12	1.05	0.016	1.034	0.07	93.8	1.4	92.4	6.2	
4	7/9	0.666	0.61	0.065	0.545	0.056	91.6	9.8	81.8	8.4	
4	7/10	0.881	0.835	0.073	0.762	0.046	94.8	8.3	86.5	5.2	
4	7/13	0.92	0.823	0.072	0.751	0.097	89.5	7.8	81.7	10.5	
4	7/15	0.41	0.36	0.068	0.792	0.05	87.8	16.6	71.2	12.2	Diabetes mellitus with gangrene of foot. Strict anti-diabetic diet. Urine contains sugar.
5	7/2	0.822	0.689	0.086	0.603	0.133	83.8	10.4	73.4	16.1	
5	7/7	0.751	0.621	0.013	0.612	0.128	82.7	1.73	81.2	15.7	
6	6/28	1.09	0.851	0.116	0.735	0.239	78.1	10.7	67.4	21.9	Diabetes mellitus. Strict anti-diabetic diet. Urine contains sugar.
6	7/1	1.34	0.936	0.088	0.848	0.404	69.8	6.5	63.3	30.1	
6	7/22	0.38	0.247	0.018	0.229	0.130	65.5	4.8	60.7	34.5	
6	7/28	0.693	0.555	0.032	0.52	0.138	80.05	4.67	75.4	19.9	Diabetes mellitus. Fifty gm. bread tolerance. Urine free from sugar.
7	6/25	1.97	1.244	0.121	1.123	0.726	63.1	6.1	57.0	36.8	
7	7/2	1.395	1.225	0.116	1.109	0.170	87.8	8.3	79.5	12.2	Diabetes mellitus. Strict anti-diabetic diet. Urine free from sugar. 125 gm. of bread added to diet. Urine contains sugar. 100 gm. of bread added to diet. Urine contains sugar. 75 gm. of bread added to diet. Urine free of sugar. 75 gm. of bread added to diet. Urine free of sugar. 100 gm. of bread added to diet. Urine contains sugar. Diabetes mellitus. Strict anti-diabetic diet. Urine contains sugar.
8	6/29	0.924	0.728	0.099	0.629	0.196	78.8	10.7	68.1	21.2	
8	7/1	0.866	0.694	0.114	0.58	0.172	80.1	13.2	66.9	19.8	
8	7/3	0.921	0.721	0.09	0.631	0.20	78.3	9.8	68.5	21.7	
8	7/5	1.06	0.83	0.011	0.819	0.23	78.3	10.4	67.9	21.7	
8	7/6	0.945	0.81	0.116	0.694	0.135	85.7	12.3	73.4	14.3	
8	7/7	0.925	0.598	0.076	0.522	0.327	64.7	8.2	56.5	35.3	
8	7/8	0.925	0.739	0.099	0.64	0.186	79.9	10.7	69.2	20.1	
8	7/9	0.98	0.752	0.084	0.668	0.227	76.8	8.6	68.2	23.2	
9	7/6	0.404	0.275	0.021	0.254	0.129	68.1	5.2	62.9	31.9	
9	7/9	0.331	0.264	0.031	0.233	0.067	79.8	9.4	70.4	20.3	Diabetes mellitus. Strict anti-diabetic diet. Urine contains sugar.
9	7/16	0.326	0.289	0.03	0.259	0.037	88.7	9.2	79.5	11.3	
9	7/18	0.372	0.30	0.03	0.27	0.072	80.7	8.7	72.0	19.3	
9	7/19	0.271	0.20	0.034	0.166	0.07	73.8	12.5	61.3	25.8	
9	7/26	0.192	0.161	0.038	0.124	0.031	83.8	19.5	64.3	16.1	



TABLE 2.—THE URINARY SULPHUR PARTITION IN CANCER

Case No.	Date.	Total Sulphur, gm.	Sulphate Sulphur, gm.	Ethereal Sulphate Sulphur, gm.	Inorganic Sulphate Sulphur, gm.	Neutral Sulphur, gm.	Sulphate Sulphur ÷ Total Sulphur, Pct.	Ethereal Sulphate S. ÷ Total Sulphur, Pct.	Inorg. Sulphate S. ÷ Total Sulphur, Pct.	Neutral Sulphur ÷ Total Sulphur, Pct.	Diagnosis and Remarks.
11	7/3	0.78	0.25	0.09	0.16	0.53	32.1	11.1	21.0	67.9	Carcinoma of cervix. Phlegmon of abdomen.
12	6/30	0.83	0.61	0.06	0.55	0.22	73.9	6.9	67.0	26.0	Carcinoma of rectum.
13	6/27	1.33	1.06	0.097	0.96	0.27	79.7	7.3	72.4	20.3	Carcinoma of stomach.
13	6/28	2.84	2.19	0.095	2.09	0.65	77.1	3.3	73.8	22.9	
14	7/29	0.50	0.34	0.05	0.297	0.155	69.0	9.6	59.4	31.0	Carcinoma of lung. Metastatic from breast.
15	7/26	0.78	0.65	0.037	0.613	0.13	83.3	4.7	78.6	16.6	Carcinoma of cervix.
15	7/27	0.82	0.68	0.073	0.614	0.14	82.9	8.9	74.0	17.1	Patient on Folin standard diet.
15	7/28	0.60	0.50	0.11	0.39	0.12	82.7	18.3	64.4	20.0	
15	7/29	0.62	0.50	0.11	0.39	0.12	80.7	17.7	63.0	19.4	
15	7/30	0.78	0.65	0.037	0.613	0.13	83.3	4.7	78.6	16.6	
16	7/8	0.60	0.49	0.047	0.446	0.11	81.7	7.7	74.0	18.2	Carcinoma of breast and pulmonary tuberculosis.
16	7/21	0.62	0.51	0.049	0.449	0.11	81.7	7.6	73.9	18.2	Cystadenoma of ovary with peritoneal metastases.
17	7/7	0.24	0.13	0.018	0.109	0.11	53.8	7.6	46.2	46.2	Carcinoma of stomach.
18	7/25	0.39	0.34	0.048	0.292	0.05	87.2	12.3	74.9	12.8	
18	7/26	0.58	0.51	0.047	0.463	0.07	87.9	8.1	79.8	12.1	
18	7/27	0.55	0.49	0.06	0.42	0.06	89.1	10.9	78.2	10.9	
18	8/9	0.78	0.68	0.096	0.58	0.10	87.2	12.3	74.9	12.8	
18	8/15	1.08	0.94	0.11	0.83	0.137	87.3	10.3	77.0	12.6	
18	8/17	0.728	0.64	.....	.....	0.086	88.2	.....	.....	11.7	
18	8/19	0.74	0.62	.....	.....	0.12	83.6	.....	.....	16.3	
18	8/22	0.67	0.61	.....	.....	0.058	91.3	.....	.....	8.6	
18	8/23	0.77	0.72	.....	.....	0.048	93.7	.....	.....	6.2	
18	8/25	1.02	0.89	.....	.....	0.125	87.6	.....	.....	12.3	
18	8/26	0.348	0.284	.....	.....	0.063	84.6	.....	.....	18.5	
18	8/27	0.616	0.534	.....	.....	0.121	86.8	.....	.....	19.6	
18	8/28	0.74	0.62	.....	.....	0.120	83.6	.....	.....	16.3	
18	8/29	0.92	0.76	.....	.....	0.16	82.9	.....	.....	17.0	
18	8/30	1.02	0.89	.....	.....	0.125	87.6	.....	.....	12.3	
19	6/21	0.995	0.753	0.101	0.65	0.24	75.6	10.1	65.5	24.4	Early carcinoma of larynx.
19	6/22	0.712	0.47	0.127	0.34	0.24	65.9	17.8	48.1	34.0	
19	6/28	1.82	1.2	0.183	0.68	0.62	65.9	10.0	55.9	34.0	
19	6/29	1.86	1.47	0.111	1.36	0.39	79.1	5.9	73.2	20.9	
19	6/30	1.44	0.67	0.111	0.56	0.76	46.8	7.7	39.1	53.2	
19	7/3	0.62	0.42	0.119	0.30	0.20	67.6	19.1	48.5	32.4	
20	8/9	0.86	0.78	0.053	.....	0.17	91.6	6.1	.....	20.0	Carcinoma of glands of neck. Operated two months ago.
21	8/9	0.61	0.50	0.04	0.46	0.11	81.9	6.5	75.4	18.1	Carcinoma of larynx and esophagus. Gastrostomy on July 29.
21	8/12	0.90	0.63	0.037	.....	0.262	70.8	4.0	.....	29.1	
21	8/14	0.49	0.41	0.032	.....	0.081	83.4	6.5	.....	16.5	
21	8/15	0.78	0.58	0.052	.....	0.26	73.5	6.2	.....	32.8	
21	8/16	0.63	0.53	0.04	.....	0.113	83.6	6.5	.....	18.0	
22	6/22	1.02	0.716	0.099	0.617	0.304	70.2	9.7	60.5	29.8	Inoperable carcinoma of stomach.
22	6/23	0.456	0.354	0.058	0.296	0.102	77.6	12.7	64.9	22.4	
22	6/27	0.81	0.61	0.123	0.486	0.198	75.4	15.2	60.2	24.5	
22	7/3	0.41	0.308	0.089	0.015	0.098	75.8	22.1	53.7	24.1	
23	6/22	0.898	0.70	0.124	0.577	0.197	78.1	13.8	64.3	21.9	Inoperable carcinoma of stomach.
23	6/23	0.814	0.587	0.052	0.535	0.227	72.1	6.4	65.7	27.9	
23	6/30	1.64	1.28	0.084	1.196	0.362	77.9	5.1	72.8	22.1	
23	7/1	2.17	1.53	0.122	0.812	0.639	70.6	5.6	65.0	29.4	

TABLE 3.—THE URINARY SULPHUR PARTITION IN VARIOUS OTHER DISEASES

Case No.	Date.	Total Sulphur, gm.	Sulphate Sulphur, gm.	Ethereal Sulphate Sulphur, gm.	Inorganic Sulphate Sulphur, gm.	Neutral Sulphur, gm.	Sulphate Sulphur ÷ Total Sulphur, Pet.	Neutral Sulphur ÷ Total Sulphur, Pet.	Ethereal Sulphate S. ÷ Total Sulphur, Pet.	Inorg. Sulphate S. ÷ Total Sulphur, Pet.	Diagnosis and Remarks.
24	6/30	1.13	0.33	0.08	0.25	0.80	29.1	6.9	22.2	70.8	Chronic interstitial nephritis. Mag. sulph. given.
25	7/24	0.812	0.68	0.06	0.62	0.132	83.7	7.3	76.4	16.2	Chronic interstitial nephritis.
26	8/8	0.66	0.56	0.059	0.50	0.095	84.7	9.5	76.2	14.4	Fracture of ribs with pneumonia.
26	8/10	0.63	0.54	0.032	0.51	0.09	85.2	5.0	80.2	14.7	
26	8/12	0.74	0.54	0.04	0.50	0.198	73.1	55.7	67.6	26.8	
26	8/18	0.86	0.72	.....	.....	0.136	83.7	.....	.....	15.7	
26	8/20	0.87	0.73	0.047	0.68	0.136	83.9	5.4	78.5	15.8	
26	8/25	0.77	0.73	.....	.....	0.04	94.8	.....	.....	5.3	
27	7/29	1.98	0.17	0.078	0.923	1.816	8.5	3.9	4.6	90.1	Lobar pneumonia.
28	8/14	1.13	1.10	0.057	1.04	0.028	97.5	5.2	92.4	2.4	Chronic lead poisoning. Mag. sulph. and potassium iodid treatment.
28	8/16	1.46	1.37	0.053	1.31	0.089	93.8	3.6	90.1	4.0	
28	8/17	1.22	1.07	0.03	1.06	0.150	87.4	2.5	86.9	12.5	
29	8/4	0.95	0.88	0.025	0.925	0.121	92.5	2.6	97.3	12.7	Chronic lead poisoning. Mag. sulph. and potass. iodid treatment.
29	8/5	1.17	1.09	0.024	1.124	0.08	93.1	20.5	96.2	7.1	
30	6/27	0.74	0.53	0.05	0.47	0.22	70.9	7.3	63.6	29.1	Bronchial asthma. Epinephrin and KI given.
31	7/5	0.28	0.22	0.02	0.20	0.06	77.5	7.1	70.4	22.5	Chronic appendicitis one week after operation.
32	7/8	0.395	0.355	0.043	0.312	0.04	89.9	10.9	79.0	10.1	Clinically gastric-carcinoma; operation showed chronic appendix.
32	7/10	0.248	0.216	0.036	0.18	0.03	87.1	14.5	72.6	12.9	Hepatic abscess.
33	7/26	0.552	0.44	0.05	0.39	0.11	79.7	9.1	70.6	19.9	Syphilitic hepatitis. Carlsbad salts given.
34	7/25	0.60	0.50	0.11	0.39	0.12	82.7	18.3	64.4	20.0	
34	7/26	1.01	0.86	0.094	0.766	0.15	85.1	9.3	75.8	14.8	Perihepatitis at operation.
35	6/21	3.36	1.19	0.143	1.05	2.17	35.4	4.3	31.1	64.6	Bile in urine. Carlsbad salts daily. Urine free from bile.
35	6/22	1.37	1.13	0.094	1.04	0.24	82.5	6.9	75.6	17.5	
36	6/28	2.86	1.84	0.12	0.73	1.02	64.4	4.2	60.2	35.5	Cholelithiasis with biliary fistula. (Slow in healing.)
36	7/3	2.80	1.89	0.12	0.87	0.91	67.5	4.2	63.3	32.5	
36	7/5	1.61	1.08	0.134	0.94	0.53	67.0	8.3	58.7	33.1	During period 6/28 to 7/6 the urine contained bile.
36	7/6	1.69	1.25	0.163	1.08	0.44	73.8	9.7	64.1	26.1	
36	7/7	1.13	1.07	0.12	0.95	0.06	94.7	10.6	84.1	5.3	
36	7/8	1.48	1.34	0.11	1.23	0.14	90.6	7.3	83.3	9.4	Urine free from bile.
36	7/9	1.28	1.09	0.09	1.00	0.19	85.2	6.8	78.4	14.8	
36	7/10	1.50	1.31	0.08	1.23	0.19	87.4	5.3	82.1	12.8	
36	7/12	1.55	1.38	0.16	1.22	0.17	89.0	10.4	78.6	10.9	
36	7/13	0.72	0.49	0.11	0.37	0.23	67.4	15.7	51.7	32.6	Urine again contained bile.
36	7/14	2.80	1.88	0.12	0.86	0.91	67.5	4.2	63.3	32.5	
37	8/17	0.68	0.60	0.033	0.57	0.08	87.9	4.8	83.1	10.6	Typhoid fever. Second week of disease.
37	8/22	0.86	0.72	0.033	0.68	0.147	83.0	3.8	79.2	16.9	
37	8/24	0.62	0.54	.....	.....	0.08	87.0	.....	.....	12.9	
37	8/25	0.49	0.41	.....	.....	0.08	82.9	.....	.....	17.03	
37	8/28	0.58	0.48	.....	.....	0.10	82.7	.....	.....	17.3	
38	7/15	0.49	0.43	0.048	0.085	0.057	88.4	9.8	78.6	11.7	Chronic myocarditis with broken compensation.
38	7/25	0.48	0.42	0.034	0.086	0.060	87.5	7.1	80.4	12.5	Codein given.
38	7/27	0.37	0.35	0.090	0.026	0.020	94.6	24.3	70.3	5.4	Hypopituitarism. Patient on standard Folin diet.
39	6/23	0.57	0.45	0.04	0.41	0.12	78.6	6.8	71.8	21.3	
39	6/26	0.41	0.30	0.03	0.27	0.11	72.5	7.2	65.3	27.4	
39	6/27	0.80	0.66	0.06	0.60	0.14	82.4	7.1	75.3	17.5	
39	6/28	0.90	0.69	0.04	0.65	0.21	76.7	4.3	72.4	23.3	
39	6/29	0.87	0.53	0.05	0.48	0.33	61.7	6.4	55.3	38.3	
39	6/30	0.96	0.67	0.04	0.63	0.30	69.2	4.0	65.2	30.8	
40	7/26	0.38	0.32	0.024	0.35	0.06	96.3	6.3	90.0	15.8	Gastroptosis and gastric dilatation.
40	7/27	0.76	0.60	0.068	0.61	0.160	78.9	8.9	70.9	21.0	
40	7/28	0.79	0.67	0.059	0.61	0.120	84.8	7.4	77.4	15.2	

In this series, consisting of seventeen cases of various diseases other than carcinoma and diabetes, with fifty-one individual estimations, the daily average excretion of total sulphur is 0.96 gm., of neutral sulphur 0.33 gm., or 34.3 per cent. of the total sulphur. If we exclude from this group a case of lobar pneumonia with an unusually high amount of total and neutral sulphur, we still find the average total sulphur excretion to be .090 gm. and the average neutral sulphur 0.24 gm., or 26.7 per cent. of the total sulphur.

### III. CONCLUSIONS

1. The lowest average total sulphur excretion (0.88 gm. per day) was found in a series of thirteen cases of carcinoma.

2. The same series showed also the lowest average neutral sulphur excretion (0.20 gm. per day).

3. The proportion of the neutral sulphur to the total sulphur in this group is considerably higher than the normal proportion of the total sulphur excreted as neutral sulphur.

4. However, the relation of the neutral sulphur to the total sulphur is still higher in the group of seventeen various diseases (not including diabetes or carcinoma). In this group we find that both the relative and absolute amounts of total sulphur and neutral sulphur to be higher than in cancer and diabetes.

5. Eight out of nine cases of diabetes studied showed an increased excretion of neutral sulphur, both in amount and in relation to the total sulphur excreted.

6. From our experience we think it is a precarious undertaking to diagnose a malignant tumor on the basis of the absolute or relative amount of the neutral sulphur excreted in the urine, or from the daily excretion of total sulphur.

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## THE REACTION OF SALOMON AND SAXL AS A DIAGNOSTIC TEST FOR CARCINOMA \*

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Salomon and Saxl<sup>1</sup> have described a reaction in the urine which they consider to be characteristic of carcinoma. The reaction depends on the supposed presence in the urine of such patients of a sulphur-containing substance, which does not yield inorganic sulphate on treatment with dilute hydrochloric acid, but which, on subsequent treatment with hydrogen peroxid, is oxidized, with liberation of inorganic sulphate. They report positive results in 70 per cent. of cases of carcinoma. They state that the reaction is frequently positive in the urine of pregnant women and in the urine of patients with cirrhosis or abscess of the liver. In a second paper<sup>2</sup> they describe the following modification of their previous method:

One hundred c.c. of the albumin-free urine are measured into a 400 to 500 c.c. beaker, 10 c.c. of hydrochloric acid (spec. grav. 1.12) are added, the contents of the beaker are heated to boiling on an asbestos-center wire-gauze and 200 c.c. of boiling water are added at once. If the specific gravity of the urine is less than 1.020, 10 c.c., if greater than 1.020, 15 c.c. of 10 per cent. barium chlorid solution are added, drop by drop. The beaker is covered with a watch-glass and kept on a boiling water-bath for six hours. After standing twenty-four hours the liquid is filtered through a double filter and refiltered into a 500 c.c. Erlenmeyer flask. Three c.c. of perhydrol (Merck's 30 per cent  $H_2O_2$ ) are added and the mixture is boiled for fifteen minutes. It is then transferred to a conical vessel and allowed to stand. The deposit of a perceptible precipitate within a few hours is regarded as a positive reaction.

According to Kaldeck,<sup>3</sup> the reaction is not characteristic of carcinoma. He found that in the urines of nine patients with carcinoma, the reaction was positive in four, negative in four and doubtful in one. Of thirty-seven urines from other patients, eight gave positive results. Of the eight, five were from tuberculous patients, out of a total of nine examined. Pribram<sup>4</sup> tested the urine of forty patients with carcinoma, five with sarcoma and forty who were free from malignant growths. Of the cancer urines, 60 per cent. gave positive reactions; of the others, only 35 per cent. He recommended the use of potassium permanganate as the

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\* Submitted for publication June 17, 1913.

\* From the Chemical Laboratory of the Montefiore Home.

1. Salomon and Saxl: *Wien. klin. Wchnschr.*, 1911, xiii, 449.

2. Salomon and Saxl: *Deutsch. med. Wchnschr.*, 1912, xxxviii, 53.

3. Kaldeck: *Wien. med. Wchnschr.*, 1911, lxi, 1652.

4. Pribram: *Wien. klin. Wchnschr.*, 1911, xxiv, 1235.



oxidizing agent because it yielded a clearer liquid in which the precipitate could more readily be detected. Murachi<sup>5</sup> weighed the barium sulphate precipitated in the test. He had urines from four patients with carcinoma and from sixteen other patients. In the former, the amount of barium sulphate obtained varied from 0.0080 to 0.0176 gm., averaging 0.0120 gm., in 100 c.c. of urine, or 0.0779 in the twenty-four-hour quantity, or 2.43 per cent. of the total sulphur. The other urines yielded from 0.0020 to 0.0080 gm., averaging 0.00485 gm. in 100 c.c., or 0.0560 gm. in the twenty-four-hour quantity, or 1 per cent. of the total sulphur. Very good results have been reported by Petersen.<sup>6</sup> Only three out of fifty-five urine patients without carcinoma gave the reaction, whereas

TABLE 1.—NORMAL URINES

Name	BaSO <sub>4</sub> from 100 c.c.		BaSO <sub>4</sub> from Twenty-Four Hour Quantity		Ratio of Sulphur Precipitated in the Test to Total Sulphur, Per Cent.
	In the Test, mg.	Total Sulphur, gm.	In the Test, gm.	Total Sulphur, gm.	
I. G. . . .	11.0	936	.....	.....	1.18
I. G. . . .	7.8	811	.....	.....	0.96
I. G. . . .	9.8	798	0.127	10.350	1.23
W. R. . . .	5.3	333	.....	.....	1.60
B. G. . . .	15.0	1901	0.078	5.205	1.50
B. G. . . .	16.7	1107	0.149	9.982	1.50
J. B. . . .	6.8	666	0.105	10.320	1.02
P. K. . . .	6.5	692	0.091	9.688	0.94
M. W. . . .	6.7	670	0.061	6.131	1.00

of nineteen urines from seventeen cases of carcinoma only two failed to do so. The patients from whom these two urines were obtained were cachectic, and Petersen believes that this may be responsible for the negative results in these cases. He does not attempt to explain how cachexia operates to cause the disappearance of a reaction supposed to be characteristic of carcinoma.

There seems to be a general agreement among these writers that the reaction, although not characteristic of carcinoma, is much more frequent in the urines of patients with carcinoma than in other urines. It should be remembered, however, that Murachi was the only one who regularly weighed the barium sulphate obtained, and that he had only four cases of carcinoma. This is very important, for a precipitate is obtained with any urine, and normal and carcinoma urines are said to differ only

5. Murachi: *Biochem. Ztschr.*, 1912, xli, 139.

6. Petersen: *Deutsch. med. Wehnschr.*, 1912, xxxviii, 1536.

in the amount of precipitate obtained. It is very difficult, indeed, to compare the amounts of barium sulphate produced under the conditions of the test without actually weighing them, for the absorbed organic material varies greatly in amount and character, but is always considerable.

At the request of Dr. S. Wachsmann, medical director of this institution, I tried this reaction in a number of urines. It soon became evident that the differences that might exist between urines from patients with and without carcinoma were too slight to be detected by simple inspection. Thereafter the precipitates obtained were weighed. The

TABLE 2.—VARIOUS PATHOLOGICAL URINES

Name	Diagnosis	BaSO <sub>4</sub> from c.c		BaSO <sub>4</sub> from Twenty-Four Hour Quantity		Ratio of Sulphur Precipitated in the Test to Total Sulphur, Per Cent.
		In the Test, mg.	Total Sulphur, mg.	In the Test, gm.	Total Sulphur, gm.	
H. B. ....	Nephritis, myocarditis	3.0	292	0.051	4.964	1.03
A. S. ....	Muscular atrophy....	5.5	693	0.039	4.751	0.80
W. ....	Levulosuria...	4.2	388	0.067	6.208	1.09
L. M. ....	Cirrhosis of liver .....	12.6	840	0.064	4.284	1.50
E. S. ....	Myelogenous leukemia...	7.4	321	0.109	4.719	2.31
M. R. ....	Diabetes.....	3.5	342	0.034	3.320	1.01
S. ....	Pulmonary tuberculosis.	3.5	438	0.049	6.139	0.80
	Pneumonia.	9.2	770	0.149	12.470	1.20

directions of Salomon and Saxl were carefully followed, the only deviations being the following:

1. A single thickness of Schleicher and Schull's No. 589 blue ribbon filter paper was used to filter the liquid after heating with acid and standing a day. The filtrates were clear.

2. After treatment with perhydrol the mixture was allowed to stand overnight and was then filtered through a Gooch filter. The precipitate and filter were washed thoroughly, ignited and weighed.

Total sulphur was determined by Benedict's method.<sup>7</sup> Most of the determinations, both of the barium sulphate precipitated by the procedure of Salomon and Saxl and of the barium sulphate obtained from the total sulphur were made in duplicate.

7. Benedict: Jour. Biol. Chem., 1909, vi, 363.

The figures given in the accompanying tables require little comment. The amount of barium sulphate precipitated in the test from normal and pathological urines varies greatly. The highest value was obtained in a normal urine and the lowest in a urine from a patient with a very extensive carcinoma, primary in an ovary. There is apparently no relation between the amount of barium sulphate precipitated in this test and the presence or absence of carcinoma.

TABLE 3.—URINES FROM CASES OF MALIGNANT NEOPLASMS

Name	Diagnosis		BaSO <sub>4</sub> from 100 c.c.		BaSO <sub>4</sub> from Twenty-Four Hour Quantity		Ratio of Sulphur Precipitated in the Test to Total Sulphur, Per Cent.
	Carcinoma of	Established at	In the Test, mg.	Total Sulphur, mg.	In the Test, gm.	Total Sulphur gm.	
S. B. ....	Stomach ....	Operation.....	4.4	260	0.055	3.221	1.69
S. B. ....	Stomach ....	Operation.....	3.4	182	0.049	2.621	1.87
R. R. ....	Ovaries .....	Autopsy.....	0.7	182	0.011	2.957	0.38
Sol. ....	Stomach ....	Operation.....	10.0	607	0.042	2.549	1.64
G. P. ....	Ovaries .....	Operation.....	3.5	417	0.041	4.800	0.86
W. F. ....	Larynx .....	Operation.....	7.0	591	.....	.....	1.18
S. R. ....	Stomach and colon .....	Clinical examination	11.2	710	.....	.....	1.58
Spiv. ....	Peritoneal cavity ....	Operation.....	16.5	678	0.201	8.272	2.43
Leit. ....	Breast .....	Operation.....	9.8	611	0.073	4.591	1.59
S. H. ....	Esophagus ..	Autopsy.....	7.1	361	0.069	3.500	1.94
I. G. ....	Mastoid ....	Operation.....	9.1	760	0.066	5.470	1.20
E. G. ....	Larynx .....	Operation.....	6.7	650	0.088	7.540	1.03
L. P. ....	Sarcoma of Lungs .....	Clinical examination	5.8	489	0.037	3.130	1.19
F. R. ....	Hip .....	Clinical examination	9.6	753	.....	.....	1.28
H. B. ....	Peritoneal cavity ....	Operation.....	6.5	636	0.066	6.420	1.02
M. A. ....	Thigh .....	X-Ray examination	9.2	297	0.078	2.520	3.10

It is true that most of the carcinoma patients were cachectic. It may be urged that this explains the discrepancy between the results here reported and those obtained by others, but this is not probable. Several urines were obtained from non-cachectic carcinoma patients. The amounts of barium sulphate obtained from these urines were not greater than those obtained from many of the normal urines. It would also be rather remarkable that the writers mentioned had obtained urines from patients with carcinoma, only comparatively few of whom were cachectic. Moreover, the value of a proposed test for carcinoma which is negative

in cachexia, one of the most characteristic symptoms of the disease, is, on this ground alone, open to serious question.

#### SUMMARY

No differences were found to exist between the urines of patients with carcinoma and other diseases and normal individuals, in the amount of barium sulphate, either absolute or relative to the total sulphur, precipitated by the procedure of Salomon and Saxl. It is therefore concluded that the test is of no value in the diagnosis of carcinoma.



## FURTHER OBSERVATIONS ON THE PROTEIN METABOLISM OF NORMAL PREGNANCY \*

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A few months ago we reported in preliminary form<sup>1</sup> the results of complete nitrogen partition studies on three carefully controlled normal cases and five pathological cases of pregnancy. We found that some instances of high ammonia could only be explained on the assumption that the bladder had been contaminated by the use of the catheter, and in one case of eclampsia we were able to demonstrate a marked fall in this constituent of the urine by the simple expedient of thoroughly washing the bladder with boric-acid solution twice in twenty-four hours. Because of the obvious importance of this source of error in judging the significance of such findings, we determined to follow up the practice of washing the bladder whenever a high amount of ammonia was encountered. In all properly conducted metabolism studies on the dog, the twenty-four-hour urines are obtained by catheter, by washing out the residual urine with sterile water and then following this with a wash of saturated solution of boric acid. It is self-evident that we should not be content with a less perfect technic in collecting urines from bed-ridden patients, if the analysis of the urine is to be trustworthy. We have, accordingly, selected a number of normal cases, some of which showed an amount of ammonia above the normal, some of them a normal amount, and placing them on constant diets, have observed the effects of a careful irrigation with a quart of boric-acid solution once or twice within an experiment day.

### THE COURSE OF THE AMMONIA OUTPUT IN NORMAL PREGNANCY

We shall present first the urinary findings of two patients who ran perfectly normal courses just before, through and just following labor, the irrigation of whose bladders caused no alteration in the ammonia findings. Such a result can only mean that the bladder was sterile to begin with, and that the use of the catheter introduced no organism capable of splitting urea. The two cases were under the constant sur-

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\* Submitted for publication June 13, 1913.

\* All of the cases here reported were patients in the maternity wards, second division Bellevue Hospital on the service of Dr. J. Clifton Edgar.

1. Murlin and Bailey: Protein Metabolism in Late Pregnancy and the Puerperium, Jour. Am. Med. Assn., 1912, lix, 1522.

veillance of a special nurse and were maintained on a diet of milk, eggs, bread and butter, potato and ice cream, containing, as shown by the tables, an approximately uniform amount of gross potential energy. The number of calories per kilogram in the tables rises after delivery for the obvious reason that the weight was constantly decreasing. The food was not analyzed, but it is apparent from the total nitrogen figures on the days when a complete twenty-four-hour urine was obtained, that the variation in nitrogen content was not very great. The influence of the total intake of protein and energy, therefore, was reasonably constant throughout.

Before considering the tables, we wish to emphasize the fact that our determinations of the ammonia in these and all other cases have been made by the most approved technic of the Folin method. Folin has recently restated<sup>2</sup> what the procedure should be. Our water-pump is of the type recommended by him, and gives sufficient pressure to produce an air current which removes all the ammonia from 10 to 20 c.c. of N/10  $\text{NH}_4\text{Cl}$  solution in one hour. This we have verified a number of times in the course of this work. But the aeration for the removal of the ammonia from urine has never been less than four hours, and often was kept up for a much longer time.

It will be seen that the ammonia nitrogen rose in both absolute and relative amounts on the day following labor for both cases (Table 1). In order to make certain that this was not due to contamination of the bladder by the catheter, we had the bladder thoroughly irrigated twice with a saturated solution of boric acid, warmed to body temperature, on the third day of collection by the catheter. The fact that the ammonia did not fall very appreciably the following day, proves that the bladder had been clean. The larger output in both absolute and relative amounts immediately following labor probably is due to the acid products, e. g., lactic acid,<sup>3</sup> formed and not completely oxidized during labor. We found no trace of acetone bodies at this time, although the total acidity was slightly higher than it had been previously.

In both cases the absolute amount of ammonia continues higher than it was before parturition. With Case 4 (Table 1) the average for the six days on which urine was analyzed previous to delivery is 0.54 gm., and for four days after delivery (neglecting the first two on account of the influence of labor), it is 0.76 gm. With Case 5 (Table 1) it is 0.61 gm. for the two full twenty-four-hour urines before delivery and 0.92 gm. for the last six days of the post-partum period (again neglecting the first two days of the puerperium). This difference between antepartum and

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2. Folin: *Jour. of Biol. Chem.*, 1912, xi, 523.

3. This sequence is known to occur in phosphorus poisoning. (Lusk: *Am. Jour. Physiol.*, 1907, xix, 461.)

TABLE 1.—METABOLISM EXPERIMENT SHOWING COURSE OF AMMONIA EXCRETION  
CASE FOUR (S). NORMAL PREGNANCY

No.	Date, 1912	Vol. c.c.	Sp. Gr.	Acidity, c.c. N/10. 10 c.c. Diluted Urine to 2,000 c.c	Albumin <sup>1</sup>	Wt., kg.	Max. Syst. Bl.-Pr.	Cal. per Kg. in Food	Total N	NH <sub>3</sub> N	Per Cent. NH <sub>3</sub> N	Remarks
1	Oct. 28-29	1920	1015	1.92	0	78.6	.....	.....	9.52	0.41	4.3	
2	29-30	1980	1020	3.2	0	.....	100	42	17.94	0.73	4.1	
3	30-31	1540	1021	3.1	0	.....	90	42	14.75	0.76	5.2	
4	Nov. 31- 1	660 <sup>2</sup>	1024	2.7	0	.....	104	42	6.39	0.36	5.7	
5	2- 3	1350	.....	2.0	.....	.....	110	42	11.87	0.53	4.5	
6	5- 6	1150	.....	1.3	.....	.....	100	42	8.12	0.42	5.1	
7	6- 7	1100	.....	2.5	.....	.....	100	36	10.92	0.73	6.5	In labor.
8	8- 9	1450	.....	3.5	.....	.....	115	44	16.80	1.19	7.0	Delivered 10 a. m. All urines by cath. on Nov. 7 and 8.
9	9-10	1450	.....	2.3	.....	.....	110	45	16.07	0.84	5.2	All urine by catheter.
10	10-11	1640	.....	3.1	.....	.....	105	52	17.52	0.88	5.0	All urine by catheter. Bladder washed.
11	11-12	1350	.....	3.0	.....	.....	107	46	12.04	0.49	4.0	Urine voided spon- taneously.
12	12-13	1650	.....	3.7	.....	70.1	100	47	19.88	0.84	4.2	Urine voided spon- taneously.

CASE FIVE (M). NORMAL PREGNANCY

1	Oct. 28-29	2260	1061	1.06	+	61.6	.....	.....	12.15	0.61	5.0	
2	29-30	700 <sup>2</sup>	1020	2.12	+	.....	138	43	5.84	0.29	5.4	
3	30-31	1750 <sup>2</sup>	1015	1.78	+	.....	124	43	4.98	0.25	5.2	
4	Nov. 31- 1	1880	1017	2.1	++	.....	108	43	11.59	0.62	5.3	Labor Nov. 3-5. Del. Nov. 5th, 3 a. m.
5	5- 6	2000	.....	2.3	++	.....	142	40	12.86	1.28	10.0	All urine by catheter.
6	6- 7	2000	.....	1.7	++	.....	138	38	11.39	0.88	7.7	All urine by catheter.
7	7- 8	1100	.....	1.8	++	.....	128	47	17.69	1.17	6.6	All urine by catheter. Bladder washed.
8	8- 9	1850	.....	2.5	++	.....	116	48	15.98	0.98	6.1	Urine spontaneously and by catheter.
9	9-10	1650	.....	2.5	+	.....	122	49	14.89	0.79	5.3	Urine spontaneously and by catheter.
10	10-11	1400	.....	3.3	+	.....	118	50	17.08	1.05	6.1	Voided spontaneously.
11	11-12	1320	.....	2.0	+	.....	114	51	16.97	0.77	4.5	Voided spontaneously.
12	12-13	1700	.....	3.2	+	52.4	125	52	16.97	0.79	4.7	Voided spontaneously.

1. Acetone and diacetic acid absent.
2. Some urine lost.

post-partum urines in general is not due to a difference in the food. It may possibly be due to the autolysis of the uterus accompanied by the liberation of acid bodies which must be neutralized by ammonia.

The resolution of the uterus results in a gradual increase in the total output of urinary nitrogen for several days, and a greater increase on the sixth day in both cases. This confirms again the observation of Longridge,<sup>4</sup> which one of us<sup>5</sup> had already confirmed in normal cases. Because of the higher total nitrogen excretion, the percentage of the ammonia nitrogen does not increase. In fact, it is even lower for Case 4 on the days which have been averaged for the absolute amount than before delivery (4.6 per cent. as against 4.8 per cent.). In Case 5 the average for the post-partum urines, not influenced by parturition, is 5.6 per cent., and for the two full days ante-partum it is 5.2 per cent. On the whole, one may say that the percentage of ammonia nitrogen in the urine is almost the same during the puerperium as immediately before delivery.

#### EFFECT OF FOUL BLADDER

Contrasted with the above results are those presented in Table 2 for Cases 6 and 7. These are both normal pregnancies, no symptoms having been noted or complained of at any time previous to or immediately following our observations. Like Cases 4 and 5, they were kept on special diets, consisting of milk, eggs, bread and butter, custard and boiled rice and containing approximately 2,100 calories. The records show that the amounts taken each day were nearly the same. Case 6 had at the beginning of our observations 2 gm. of ammonia nitrogen in the urine. This fell gradually for about a week, when it had reached the level shown on the first day of the table. The striking thing about the chart, however, is the enormous fall in the ammonia nitrogen which resulted from washing the bladder on the second day following delivery. The interpretation of the rest of the chart must obviously be made in the light of this effect. Microscopical examination of the urines obtained before delivery, but made subsequent to the discovery of the contamination, revealed the presence of many bacteria and some pus cells, but it was a case which, without the confirmatory evidence of the effects of irrigation, might have been allowed to pass as an "unexplained" high ammonia.

No doubt a part of the high ammonia on the day of delivery and the day after may be ascribed, as we have just seen, to the effects of labor itself, but so great a fall—from 2.27 gm. ammonia nitrogen to 1.33 gm.—can only be attributed to the cleansing which the bladder received. It happens that the total nitrogen in the urine October 2 to 3 and October 4 to 5 is nearly the same, so that the fall to 9 per cent. in this total in the

4. Longridge: *Jour. Obst. and Gynecol.*, 1908, xiii, 420.

5. Murlin: *Surg., Gynecol. and Obst.*, January, 1913, p. 43.



ammonia fraction cannot be referred to a change in the diet, nor, since the autolysis is not yet far enough advanced, to a change in the uterus. That the extra ammonia came largely if not wholly from the decomposition of urea is shown by the sums of the urea and ammonia fractions for these days; and that the decomposition took place in the bladder and not in the bottle we are certain, because of the fact that the preservative

TABLE 2.—EXPERIMENTS ILLUSTRATING INFLUENCE OF FOUL BLADDER  
CASE SIX (R). NORMAL PREGNANCY

No.	Date, 1912	Volume	Sp. Gr.	Acidity	Albumin <sup>1</sup>	Total N	Urea N	NH <sub>3</sub> N	Per Cent. Urea N	Per Cent. NH <sub>3</sub> N	Diet and Condition of Patient
1	Sept. 27-28	1670	.....	1.30	Tr.	12.37	9.29	1.78	75.1	14.3 <sup>2</sup>	Special diet of milk, eggs, bread and butter, custard and boiled rice, containing about 2,100 calories.
2	28-29	1380	.....	0.80	Tr.	9.72	7.48	1.10	76.8	11.3	
3	28-29	1530	.....	2.0	....	13.00	10.04	1.35	77.2	10.3	
4	Oct. 2-3	1860	1021	1.9	....	14.71	10.60	2.27	72.0	15.4	Baby born Oct. 2. Catheter used six hours. Bladder washed.
6	4-5 <sup>3</sup>	1230	1023	0.3 <sub>2</sub>	0	14.08	10.56	1.33	75.0	9.4	
7	5-6	950	1020	1.7 <sub>5</sub>	0	12.41	9.45	1.08	76.4	8.7	

CASE SEVEN (T). NORMAL PREGNANCY

1	24-25	1800	.....	2.3	0	13.38	10.58	1.07	79.0	8.0	Third day postpartum; second day of using catheter. Special diet of milk, eggs, bread and butter, custard and boiled rice containing about 2,100 calories. Bladder washed.
2	25-26	950	.....	1.4	0	11.39	9.20	0.82	80.7	7.2	
3	26-27	1430	.....	0.8	0	13.34	10.52	0.68	78.8	5.1	

1. Acetone and diacetic acid absent.

2. In this case bacteria were found in urine before parturition.

3. The urine for October 3-4 was alkaline and is therefore not considered.

was placed in the bottle before it left the laboratory each day and because of the care which was constantly exhibited by the nurse who had charge of these cases.

Case 7 will be readily understood from the table. The fall neither in absolute nor in percentage amounts in this case is so great when the bladder was washed as it was in Case 6, but there seems to be no other explanation; for the total nitrogen in the urine was the same, and there were no unusual acid bodies in the urine which we could detect.

We have included only these two cases which showed a fall in the urinary ammonia, because they were the only ones which exhibited the same total nitrogen before and after irrigation. Several other cases were observed in which the effect of irrigation was evident. It would be foolish to assert that no clean urine can be obtained from parturient patients without washing the bladder, but it is clear from our experience that *this possible source* of contamination must now be placed alongside of others (passage of the urine over the vulva, mixture with feces, lochia, etc.), which have always been recognized. We believe the safest and surest way to avoid all dangers of contamination is the manner of collection which we have adopted, namely, drawing the urine by catheter and placing it directly into the bottle containing preservative, and washing the bladder thoroughly with boric-acid solution twice in twenty-four hours immediately following catheterization. The boric-acid wash should not be mixed with the urine, because it interferes with certain determinations. A perfectly exact termination of the twenty-four-hour period is only thus obtainable, for by suitable manipulation of the catheter the bladder can be emptied more completely than is usually possible by the voluntary efforts of a parturient woman. A first wash with sterile distilled water is advisable, in order to obtain the last traces of urine in the bladder. The procedure should be so timed that it terminates on the last moment of the twenty-four-hour period.

While this routine may seem to some unnecessary, we are certain from long experience in metabolism experiments with animals that it is the only scientific procedure in metabolism studies with bed-ridden patients.

#### COMPLETE NITROGEN PARTITIONS

Tables 3, 4 and 5 contain the complete analyses for the three normal cases whose metabolism was reported in summary in the previous paper. Several points of interest may be seen in the day-to-day record. For example, the influence of the changes in diet from "ordinary hospital fare" containing meat to a diet consisting largely of milk, can be seen especially in the columns for ammonia, creatin and total purin nitrogen; and the variation in all the fractions which occur normally, or the range of error, as the case may be, is exhibited.

#### METHODS OF ANALYSIS

Total nitrogens were determined by the Kjeldahl method, using Folin's sulphate mixture for hydrolysis; urea-plus-ammonia by Benedict's<sup>6</sup> method; ammonia as already stated; creatinin and creatin by Folin's colorimetric method, converting creatin to creatinin in 500 c.c. flasks provided with pipet condensers and kept on the electric stove just

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6. Benedict: Jour. of Biol. Chem., 1911, viii 405.

below boiling for three hours; total purin by a modification of Hall's<sup>7</sup> sedimentation method, which is in principle the old Camerer-Arnstein method of precipitation with silver; and amino-acids by a modification of the "new" formalin titration method introduced by Henriques and Sørensen.<sup>8</sup> Only the two last-named methods require any comment.

#### TOTAL PURIN METHOD

We began with Hall's purinometer, but owing to the fact that the purinometer can be used for only one determination in twenty-four hours, if results at all accurate are to be obtained, we have adapted the method to the use of the centrifuge as follows: 14 c.c. of urine (the twenty-four-hour urine diluted to 2,000 c.c.), is placed in a graduated centrifuge tube and 2 c.c. of Hall's purinometer, solution No. 1, is added and the resulting precipitate centrifuged. From the supernatant fluid 11.43 c.c. (equals 10 c.c. urine) is then measured into another centrifuge tube and 2 c.c. of strong ammonia and 2 c.c. of 3 per cent. silver nitrate solution are added. This mixture should stand for one-half hour or more to allow complete reaction. A centrifuge running at 1,800 revolutions per minute will throw down all the silver-purin in about twenty minutes. This precipitate is then stirred up with a glass rod and is washed twice with a 1 per cent. solution of ammonia, being thrown down each time by running the centrifuge for about five minutes. Finally it is washed into a Kjeldahl flask, the ammonia is boiled off with magnesium oxid and the regular Kjeldahl procedure follows for direct determination of the nitrogen. Titration is best accomplished with N/50 NAOH solution.

With a six-arm centrifuge the method is fairly rapid and is certainly not less accurate than the original Camerer-Arnstein method or any of its many earlier modifications. This we have satisfied ourselves of by use of pure uric acid, xanthin and hypoxanthin.

#### AMINO-ACID METHOD

The amino-acid fraction was determined at first by only a slight modification of the so-called "new" method of Henriques and Sørensen. This modification consisted only in driving off the ammonia by the aeration method of Folin, running the most rapid current of air obtainable with a suction pump (nearly always over night, eighteen hours). Controls with  $\text{NH}_4\text{Cl}$  solution in equal volume of water (50 c.c.) convinced us after a time that we were not getting quite all the ammonia especially in urines containing much of it, and this conclusion led us to adopt a different method of removing the ammonia; namely, by precipitation with phosphotungstic acid.<sup>9</sup>

The method which we now use is as follows: Take 200 c.c. of urine and add an equal volume of 10 per cent. phosphotungstic acid in 2 per cent. HCl. Allow to sediment for at least four hours (better over night). Pour off 200 c.c. of the supernatant fluid into a 250 c.c. flask, add 1 c.c. of 1 per cent. phenolphthalein and saturated solution of barium hydrate, or barium hydrate in substance, until the solution turns decidedly pink. Make up to 250 c.c. with water, stopper, shake up thoroughly and let stand for two hours. Filter off two 100 c.c. samples (equals 40 c.c. urine each) for duplicate titrations, and follow the regular Henriques and Sørensen procedure observing all precautions.<sup>10</sup>

7. Hall, I. Walker: *The Purin Bodies of Foodstuffs*, 1903, p. 149.

8. Henriques and Sørensen: *Ztschr. f. physiol. Chemie*, 1910, lxiv, 120.

9. Benedict and Murlin: *Proc. Soc. for Exper. Biol. and Med.*, 1912, ix, 109.

10. For a good critique of this method see "Die Formoltitration," by H. Jessen-Henson in *Abderhalden's Arbeitsmethoden*, 1913, vi, 262.

## DISTRIBUTION OF THE NITROGEN IN THE URINE OF NORMAL PREGNANCY

The data regarding the blood-pressures, temperatures, pulse and respiration in Tables 3, 4 and 5 should be convincing evidence, when taken in connection with the absence of symptoms, that these three cases were entirely normal in every respect. All of the patients were in the last month of pregnancy. They were placed in a separate ward under the care of a nurse whose main occupation was the supervision of their diets and the collection of their urines. The women did no work and were practically in a condition of rest during the course of these observations.

On the first two days and the fourteenth or last day of the observation period, they were kept on a regular ward diet which contained meat. From the third to the ninth day inclusive they received a diet of whole milk, bread and butter, and a constant amount of milk-sugar. An effort was made to keep the calories supplied proportional to the requirements of the individuals; but in this we did not wholly succeed. The requirements for women in the last few weeks of pregnancy, who are resting quietly in bed, has been found by Carpenter and Murlin,<sup>11</sup> by means of both the direct and the indirect methods of calorimetry, to be in the neighborhood of 30 calories per kilogram. For a fat woman it would not be more than 27 calories per kilogram.

We estimated the requirement for these patients sitting up part of the time and moving about the ward, to be not over 35 calories per kilogram. Only one (Case 2) of the three took that quantity of food for any length of time, and is the only one that shows a distinct gain in weight. But for the severe catharsis given on December 16, the patient would probably have finished the two-weeks period a couple of pounds heavier than at the start. That all of the patients were fairly well nourished, however, and that the children did not suffer, is shown by the fact that within three weeks all came to parturition and delivered children weighing as follows: Case 1, 9 pounds, 4 ounces; Case 2, 9 pounds, 8 ounces; Case 3, 7 pounds, 2 ounces.

In our preliminary paper and in a more recent contribution<sup>5</sup> by one of us, the general physiological significance of the different nitrogen fractions has been sufficiently considered. The questions that require further evidence and discussion are: *To what extent does pregnancy of itself alter the distribution of the nitrogen, and can such alterations be accounted for on purely physiological grounds?*

To answer the first question with any approach to accuracy it is necessary to compare the metabolism between the pregnant and the non-pregnant states either in the same individual or in different individuals

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11. Carpenter and Murlin: The Energy Metabolism of Mother and Child Just Before and Just After Birth, *THE ARCHIVES INT. MED.*, 1911, vii, 184.



in the same general health, age, etc.; and in either case on the same diet. It is desirable that the nitrogen balance should be known so that the influence of nitrogen retention may be observed. For the dog these conditions have been rigorously met by following the metabolism on the same diet throughout the entire gestation period.<sup>12</sup> For the woman, in addition to our studies, a paper by Landsberg<sup>13</sup> has appeared recently in which the author compares the metabolism as to the urea, ammonia and amino-acid nitrogens of six non-pregnant women with that of ten normally pregnant women, all kept on similar diets. Nitrogen, sulphur and phosphorus balances were kept. Heynemann<sup>14</sup> has done the same, omitting the balance studies, for the creatin and creatinin. Falk and Hessky<sup>15</sup> a short time before had made analyses for ammonia, amino-acid and polypeptid nitrogens in the urines of both pregnant and non-pregnant women, but their conditions were apparently not so carefully controlled.

We believe that we now have in these researches, together with our own, sufficient data to enable one to forecast pretty definitely for the last weeks of a normal pregnancy (for a woman whose intake of food is known and is sufficient to meet her requirements), what the distribution of the nitrogen should be.

#### UREA NITROGEN

It is now demonstrated<sup>5, 12</sup> that the retention of nitrogen in pregnancy will cause a diminution in the absolute amount of urea excreted. Both in dogs and in women it is found that where retention of nitrogen is known to be greater, or where the conditions for nitrogen retention are known to be present, the urea falls. The percentage fall is even greater than the absolute fall, because, as we shall see later, certain other nitrogen constituents of the urine are slightly increased.

Landsberg's experiments unfortunately are largely invalidated so far as the urea determinations are concerned by the method employed; namely, taking the total nitrogen in the filtrate after removal of ammonia and precipitation with alcohol and ether (Spiro's method). Multiplying this amount of nitrogen by 15/7 is said to give the urea nitrogen. This method has long been known<sup>16</sup> to yield all of the creatinin and hippuric acid nitrogen, part of the uric acid and probably a large part of the creatin nitrogen (which is nearly always present in the urine of late pregnancy), in addition to the urea nitrogen. Landsberg's estimate of the fraction (82 to 86 per cent.) is therefore certainly too high, possibly as much as 6 or 7 per cent. However, even by this method, he finds the

12. Murlin: *Am. Jour. Physiol.* 1911, xxviii, 422.

13. Landsberg: *Ztschr. f. Geburtsh. u. Gynäk.*, 1912, lxxv, 163.

14. Heynemann: *Ztschr. f. Geburtsh. u. Gynäk.*, 1912, lxxv, 110.

15. Falk and Hessky: *Ztschr. f. klin. Med.*, 1910, lxxi, 261.

16. Howe and Hawk: *Comparative Tests of Spiro's and Folin's Methods for the Determination of Ammonia and Urea.* *Jour. Biol. Chem.*, 1909, v, 477. See also Neubauer and Huppert's *Analyse des Harns*, 1910, p. 574.

average percentage for normal pregnancy (83.6 per cent.) lower than for normal non-pregnant women (85.02 per cent.).

The three normal cases here reported show an average of 77.8 per cent., 70.8 per cent. and 81.4 per cent., respectively. (Tables 3, 4 and 5.)<sup>17</sup> The lowest percentage occurs in the urine of the patient (Case 2), who received most nearly her requirement of food, who gained most in weight and was therefore probably retaining most nitrogen. The next lowest occurs in the patient (Case 1), who received the medium amount of food; and, as the figures stand, the highest percentage occurs in the case (3) that received the lowest amount of food per unit of weight. It is probable, however, that the average for Case 3 is too high (see note at bottom of table).

The same relationship holds also for the urea-plus-ammonia nitrogen, i. e., for all the nitrogen that comes from the end-products of protein digestion or from putrefaction in the alimentary canal. Such a relationship would not hold if the factor of undernutrition, which Folin<sup>18</sup> has shown to play an important part in determining the percentage of urea, ammonia, etc., entered here.

The urea average for the whole antepartum period, taking the three cases together, is 76.6 per cent. For the three cases reported by Murlin<sup>5</sup> it was 75.6 per cent. The range in all six cases is from 70.8 (Case 2 this series) to 79.4 (Case 1 former series). Taking urea and ammonia together or "total-amid nitrogen" the average for this series is 84.2 per cent.; for the former series 82.5 per cent. The range is from 80.3 (Case 2 this series) to 86.7 (Case 3 former series).

#### AMMONIA NITROGEN

These tables show something of the range that may be expected in the ammonia excretion from normal cases four to six weeks before delivery. Case 1 shows an average of 0.74 gm., 6.9 per cent. of the total nitrogen; Case 2, 0.54 gm., 10.3 per cent. of the total, and Case 3, 0.51 gm., or 5.3 per cent. of the total. Landsberg finds as the results of about four days' examination on ten patients that the average of all is 0.786 gm. ammonia nitrogen, or 6.8 per cent. as contrasted with 0.771 gm., or 4.3 per cent. from six non-pregnant women on a similar diet. Landsberg does not lay special stress on the fact that the absolute amount is but slightly higher and that the difference in percentage is really due to the larger output of total nitrogen (16.03 gm. in the non-pregnant subject, as compared with 12.68 gm. in the pregnant). Falk and Hessky, among the later observers, also found the relation of the ammonia nitrogen to the total nitrogen "higher than the figures which are assumed to be normal on a

17. The averages in these tables differ slightly from the averages published in our preliminary paper because three more days are here included.

18. Folin: *Am. Jour. Physiol.*, 1905, xiii, 66.

TABLE 3.—RESULTS OF METABOLISM EXPERIMENT IN

No.	Date, 1911	Condition of Patient					Diet	Qualitative Examination				
		Weight, kg.	Blood-Pr.	Temperature	Pulse	Respiration		Cal. per kg.	Volume	Spec. Grav.	Albumin	React. N/10 NaOH
	Dec.											
1	5-6	.....	.....	97.8	108	22	Ordinary hospital fare...	.....	1050	1031	Tr.	Ac. 5 c.c.
2	6-7	.....	115	97.8	88	22	Ordinary hospital fare...	.....	980	1032	Tr.	Ac. 5 c.c.
3	7-8	.....	120	97.8	92	22	Milk, bread and butter..	18	768	1034	0	Ac.
4	8-9	75.0	118	97.8	96	22	Milk, bread and butter..	24	800	1035	0	Ac.
5	9-10	75.0	116	98.0	92	22	Milk, bread and butter..	20	860	1035	0	Ac. 6 c.c.
6	10-11	75.0	110	98.0	96	24	Milk, bread and butter..	29	830	1030	0	Ac. 6 c.c.
7	11-12	74.5	96	98	96	24	Milk, bread and butter..	33	750	1033	0	Ac. 9 c.c.
8	12-13	75.4	108	98	88	24	Milk, bread and butter <sup>3</sup> ..	37	930	1034	0	Ac. 6.8 c.c.
9	13-14	75.4	108	97.8	92	24	Milk, bread and butter <sup>3</sup> ..	36	1085	1030	0	Ac.
10	14-15	75.0	118	98	92	24	Milk and starchy foods <sup>2</sup> .	31	1250	1028	0	Ac.
11	15-16	74.8	118	97.8	104	22	Milk and starchy foods <sup>2</sup> .	31	585	1026	0	Ac.
12	16-17	75.0	114	98	96	22	Milk and starchy foods <sup>2</sup> .	31	635	1028	0	Ac.
13	17-18	75.0	110	98	102	24	Milk and starchy foods <sup>2</sup> .	31	1010	1026	0	Ac.
14	18-19	74.8	110	98	104	24	Ordinary hospital fare...	.....	1360	1021	0	Ac.

Average

1. Urine bottle broken.

2. Oatmeal, bread, graham crackers, baked potato.

TABLE 4.—RESULTS OF METABOLISM EXPERIMENT IN

No.	Date, 1911	Condition of Patient					Diet	Urine Qualitative Examination				
		Weight, kg.	Blood-Pr.	Temperature	Pulse	Respiration		Cal. per kg.	Volume	Spec. Grav.	Albumin	Reaction
	Dec.											
1	5-6	.....	.....	97.6	116	24	Ordinary hospital fare.....	.....	1550	1016	Tr.	Ac. <sup>1</sup>
2	6-7	.....	130	97.8	112	22	Ordinary hospital fare.....	.....	960	1016	0	Ac.
3	7-8	.....	140	98	110	22	Milk, bread and butter.....	24	932	1020	0	Ac.
4	8-9	65.4	115	98	112	22	Milk, bread and butter.....	28	812	1018	Tr.	Ac.
5	9-10	65.0	112	97.8	112	22	Milk, bread and butter.....	21	700	1020	Tr.	Ac.
6	10-11	65.0	106	97.8	128	32	Milk, bread and butter.....	26	710	1020	0	Ac.
7	11-12	65.0	105	98	100	24	Milk, bread and butter.....	27.5	540	1017	0	Ac.
8	12-13	65.9	112	98.2	96	24	Milk, bread and butter <sup>4</sup> .....	38.3	740	1018	Tr.	Ac.
9	13-14	65.9	114	98	88	24	Milk, bread and butter <sup>4</sup> .....	33.5	650	1020	Tr.	Ac.
10	14-15	65.7	116	98.2	84	22	Milk and starchy foods <sup>2</sup> .....	35	750	1018	Tr.	Ac.
11	15-16	65.7	122	98	112	24	Milk and starchy foods.....	35	610	1026	0	Ac.
12	16-17	65.9	118	98.2	100	22	Milk and starchy foods.....	35	420 <sup>3</sup>	1024	0	Ac.
13	17-18	65.0	106	98	96	22	Milk and starchy foods.....	35	720	1022	Tr.	Ac.
14	18-19	65.4	110	98.2	100	22	Ordinary hospital fare.....	.....	1130	1014	Tr.	Ac.

Average

1. Acetone and diacetic acid absent throughout.

2. Oatmeal, bread, graham crackers, baked

NORMAL PREGNANCY. CASE 1 (B. H.) NINTH MONTH

Quantitative Urine Analysis of Nitrogen Fractions														
Total N, gm.	Urea N, gm.	NH <sub>3</sub> N, gm.	Cr <sub>1</sub> N, gm.	Cr <sub>2</sub> N, gm.	Total Purin N gm.	Amino Acid N, gm.	Undet. N, Gm.	Urea N, Per Cent.	NH <sub>3</sub> N, Per Cent.	Cr <sub>1</sub> N, Per Cent.	Cr <sub>2</sub> N, Per Cent.	Total Purin N Per Cent.	Amino Acid, N Per Cent.	Undet. N, Per Cent.
15.06	11.43	0.83	0.41	0.13	0.22	.....	.....	75.9	5.5	2.7	0.9	1.4	.....	.....
12.88	9.90	0.74	0.35	0.19	.....	0.37	.....	76.9	5.7	2.7	1.5	.....	2.9	.....
11.54	8.91	0.78	0.39	0.15	0.41	0.40	0.42	77.2	6.8	3.4	1.3	4.2	3.5	3.6
10.86	8.73	0.68	0.37	0.14	0.31	0.43	0.20	80.4	6.3	3.4	1.3	2.8	4.0	1.8
11.37	8.76	0.71	0.38	0.06	0.25	0.34	0.88	77.0	6.2	3.3	0.5	2.2	3.0	7.8
11.28	8.70	0.71	0.34	0.07	0.28	0.35	0.92	77.1	8.1	3.0	0.6	2.4	3.1	8.1
12.07	9.27	0.88	0.40	0.08	0.28	0.50	0.66	76.8	9.4	3.3	0.7	2.3	4.1	5.4
12.43	9.48	0.88	0.36	0.09	0.36	0.45	0.81	76.3	7.1	2.9	0.7	2.9	3.6	6.5
13.33	10.54	0.88	0.38	0.05	.....	0.47	.....	79.2	6.6	2.9	0.4	.....	3.6	.....
15.57	12.99	0.78	0.41	0.16	0.64	0.53	0.04	83.4	5.0	2.6	1.3	4.1	3.4	0.2
9.02	7.21	0.57	0.27	0.19	.....	0.59	0.19	80.0	6.3	3.0	2.2	.....	6.5	2.1
8.06	6.25	0.58	0.28	0.06	0.36	0.47	0.06	77.5	7.2	3.5	0.7	3.5	5.8	1.6
10.98	8.17	0.71	0.32	0.20	0.31	0.63	0.64	74.4	6.5	2.9	1.8	2.8	5.7	2.9
8.29	6.45	0.83	0.38	0.09	0.67	0.61	0.75	77.8	10.0	4.6	1.1	8.1	7.4	9.0
11.61	9.30	0.74	0.38	0.12	0.37	0.47	0.55	77.8	6.9	3.2	1.1	3.3	4.4	4.4

3. Fifteen gr. thyroid extract daily.

NORMAL PREGNANCY. CASE 2 (M. L.) NINTH MONTH

Quantitative Urine Analysis of Nitrogen Fractions														
Total N, gm.	Urea N, gm.	NH <sub>3</sub> N, gm.	Cr <sub>1</sub> N, gm.	Cr <sub>2</sub> N, gm.	Total Purin N gm.	Amino Acid N, gm.	Undet. N, Gm.	Urea N, Per Cent.	NH <sub>3</sub> N, Per Cent.	Cr <sub>1</sub> N, Per Cent.	Cr <sub>2</sub> N, Per Cent.	Total Purin N Per Cent.	Amino Acid, N Per Cent.	Undet. N, Per Cent.
9.01	6.52	0.76	0.42	0.0	0.75	0.29	0.27	72.3	8.4	4.7	0.0	8.3	3.2	3.0
6.49	4.90	0.51	0.27	0.05	0.43	0.15	0.18	76.5	7.9	4.2	0.7	3.0	2.4	2.8
6.49	4.72	0.65	0.35	0.06	0.34	0.28	0.09	72.7	10.0	5.4	0.9	5.2	4.3	1.4
5.45	4.02	0.51	0.28	0.11	0.25	0.26	0.03	73.7	9.3	5.1	2.0	4.6	4.8	0.4
4.65	3.28	0.47	0.24	0.0	0.22	0.37	0.07	70.6	10.1	5.2	0	4.7	7.9	1.5
5.71	4.17	0.53	0.28	0.0	0.08	0.28	0.37	72.8	9.3	4.9	0	1.4	4.9	6.0
3.42	2.38	0.36	0.14	0.0	0.22	0.18	0.14	69.7	10.5	4.1	0	6.4	5.3	4.0
5.38	3.91	0.60	0.17	0.0	0.25	0.24	0.21	72.6	11.2	3.2	0	4.6	4.4	3.9
4.81	3.35	0.59	0.16	0.13	0.20	0.20	0.18	67.6	12.2	3.3	2.7	4.2	4.2	3.7
5.10	3.55	0.54	0.22	0.11	0.28	0.35	0.05	69.6	10.6	4.3	2.2	5.5	6.9	0.9
5.82	4.13	0.57	0.21	0.0	0.31	0.20	0.41	70.9	9.8	3.6	0.0	5.3	3.4	7.0
3.36	2.11	0.58	0.23	0.0	0.17	0.26	0.11	63.8	17.3	3.9	0	5.1	7.7	3.3
5.71	3.98	0.50	0.22	0.0	0.31	0.26	0.44	69.2	8.8	3.9	0	5.4	4.6	7.7
5.43	3.70	0.44	0.23	0.0	0.20	0.35	0.51	68.2	8.1	4.2	0	3.7	6.4	8.4
5.49	3.91	0.54	0.24	0.03	0.29	0.26	0.22	70.8	10.3	4.3	0.6	4.9	5.0	3.9

potato. 3. Catharsis. 4. Fifteen gr. thyroid extra daily.



mixed diet (3 to 5 per cent.),” and their table shows many instances of a percentage higher than any that we have found except on a day of catharsis (see table).

Since the liver is the organ especially charged with the conversion of ammonia to urea, particularly that ammonia which may arise from putrefactive processes in the large intestine,<sup>19</sup> it has been assumed that a high ammonia, unaccounted for by any unusual acid bodies in the urine, is a sign of liver inefficiency,<sup>20</sup> and Falk and Hessky adopt this explanation of their findings, pointing out that the intolerance of the pregnant woman for levulose is a parallel indication.

TABLE 5.—RESULTS OF METABOLISM EXPERIMENT IN

No.	Date, 1911	Weight, kg.	Blood-Pr.	Temperature	Pulse	Respiration	Diet	Cal. per kg.	Urine Qualitative Examination				
									Volume	Spec. Grav. <sup>1</sup>	Albumin	React. N/10 NaOH	10 c.c. of Urine Diluted to 2,000 c.c.
1	Dec. 5-6	.....	.....	98	100	22	Ordinary hospital fare.	.....	1700	1028	0 <sup>2</sup>	{ 28 c.c. Ac.	
2	6-7	.....	97	98	108	24	Ordinary hospital fare.	.....	1470	1020	0		
3	7-8	.....	117	98	112	24	Milk, bread and butter.	18	722	1032	0	Ac.	
4	8-9	83.9	110	97.8	100	24	Milk, bread and butter.	20	612	1034	0	Ac.	
5	9-10	83.6	115	98.2	108	22	Milk, bread and butter.	19	570	1030	0	6.4 c.c.	
6	10-11	83.1	105	97.4	100	24	Milk, bread and butter.	23	1330	1020	0	3.5 c.c.	
7	11-12	83.4	90	98	96	24	Milk, bread and butter <sup>3</sup>	27	780	1027	0	6.0 c.c.	
8	12-13	84.1	96	97.8	92	24	Milk, bread and butter <sup>3</sup>	29	780	1026	0	6.1 c.c.	
9	13-14	83.9	100	98.2	96	24	Milk, bread and butter.	30	750	1022	0	Ac.	
10	14-15	83.6	106	97.8	96	24	Milk and starchy foods <sup>4</sup>	27.4	760	1022	0	Ac.	
11	15-16	83.4	94	97.8	100	24	Milk and starchy foods	27.4	800	1028	0	Ac.	
12	16-17	83.6	94	98.4	100	24	Milk and starchy foods	27.4	667	1024	0	Ac.	
13	17-18	84.1	98	98.4	100	22	Milk and creatin foods	27.4	670 <sup>3</sup>	1022	0	Ac.	
14	18-19	84.1	98	98.2	96	24	Ordinary hospital fare.	.....	1140	1014	0	Ac.	

Average

1. Acetone, albumin and diacetic acid absent. 2. Error probably on urea. 3. Some urine lost.

Heynemann, however, reviews all the signs of liver inefficiency which have been accepted by different observers, including urobilinuria, faulty fat metabolism, levulosuria, etc., and reaches the conclusion that none of these have been established. He cites, for example, work tending to show that all female subjects have a lower tolerance for levulose than have male subjects with whom the pregnant have so often been compared. Landsberg seems to dispose of the high ammonia as a sign of liver ineffi-

19. Folin: Jour. Biol. Chem., 1912, xi, 161.

20. See, for example, Ewing and Wolff: Amer. Jour. Obst., 1907, lv, 289.

ciency by showing that the ammonia runs parallel to the total acidity in such urines. The excess acid he thinks may originate in the fetus.

While we are quite prepared to admit the possibility of Landsberg's explanation, it must be remembered that he has in his own figures and we have in ours only a slight discrepancy in the absolute amount of ammonia nitrogen to be accounted for. As a matter of fact, we do not regard our absolute ammonia figures as high at all. *It is only the relative or percentage amount which is high and that is partly explained at once by the lower total nitrogen excretion.* Assuming, however, that this is

NORMAL PREGNANCY. CASE 3 (E. B.) NINTH MONTH

Quantitative Urine Analysis of Nitrogen Fractions

Total N, gm.	Urea N, gm.	NH <sub>3</sub> N, gm.	Cr <sub>1</sub> N, gm.	Cr <sub>2</sub> N, gm.	Total Purin N gm.	Amino Acid N, gm.	Undet. N, Gm.	Urea N, Per Cent.	NH <sub>3</sub> N, Per Cent.	Cr <sub>1</sub> N, Per Cent.	Cr <sub>2</sub> N, Per Cent.	Total Purin N, Per Cent.	Amino Acid, N Per Cent.	Undet. N, or Error, Per Cent.
14.28	12.58	0.53	0.43	0.22	0.50	0.27	-0.25	88.1	3.7	3.0	1.6	3.5	1.9	-1.8
11.20	8.90	0.50	0.38	0.19	0.31	0.22	-0.70	79.5	4.5	3.4	1.7	2.8	2.0	6.2
10.37	9.18	0.50	0.40	0.17	0.59	0.31	-0.78	88.5	4.8	3.9	1.6	5.7	3.0	-7.5 <sup>a</sup>
10.64	8.61	0.46	0.39	0.12	0.12	0.29	0.65	80.8	4.3	3.7	1.1	1.1	2.7	6.1
8.06	6.54	0.34	0.25	0.08	0.31	0.24	0.30	81.0	4.2	3.1	0.8	3.7	3.0	3.7
11.84	9.31	0.57	0.42	0.08	0.22	0.39	0.85	78.6	4.8	3.5	0.7	1.8	3.3	2.2
10.15	8.10	0.53	0.33	0.04	0.45	0.27	0.43	79.8	5.2	3.2	0.4	4.4	2.6	4.2
10.70	9.34	0.63	0.36	0.10	0.31	0.29	-0.33	87.3	5.9	3.4	0.9	2.9	2.7	-3.1
7.95	6.44	0.45	0.26	0.08	0.59	0.24	-0.11	81.0	5.7	3.3	1.0	7.4	3.0	-1.4
10.25	8.13	0.66	0.38	0.08	0.25	0.38	0.37	79.3	6.4	3.7	0.8	2.4	3.7	3.6
11.03	8.57	0.66	0.41	0.02	0.50	0.45	0.40	77.7	6.0	3.7	0.2	4.5	4.0	3.6
8.47	6.98	0.50	0.31	0.12	0.22	0.44	-0.10	82.4	6.1	3.7	1.4	2.6	5.9	-1.2
5.88	4.56	0.36	0.20	0.18	0.20	0.29	+0.09	72.6	6.1	3.4	3.0	3.4	4.9	1.6
6.61	5.17	0.43	0.23	0.06	0.25	.....	.....	78.2	6.5	3.5	0.9	3.8	.....	.....
9.82	8.03	0.51	0.34	0.11	0.34	0.31	+0.16	81.4	5.3	3.5	1.2	3.6	3.3	+1.6

4. Oatmeal, bread, graham crackers, baked potato.

3. Fifteen gr. thyroid extract daily.

not sufficient explanation, is it not possible that the higher ammonia is due to a high relative (not high absolute) acidity? Suppose the fetus were drawing bases from the maternal blood more rapidly than acids, the effect would be to increase the ammonia in the urine of the mother, just as is known to occur when an excess of bases is excreted through the bowel.<sup>21</sup>

21. Cf. Steinitz: Jahrb. f. Kinderh., 1903, lvii, 689.

21a. Hoffström [Skand. Archiv. f. Physiol., 1910, xxiii, p. 326] indeed has shown that the fetus lays claim to the calcium of the food much more rapidly than to the sulphur and phosphorus.

## INFLUENCE OF CATHARSIS ON AMMONIA PERCENTAGE

On December 16 two of the three patients were given calomel, salts and high colonic irrigation for the purpose of preparing the bowel for the administration of creatin the next day (see below). The tables show that the volume of urine was much reduced in Case 2, a fact which we have ascribed to the reduced absorption from the bowel. The amount of creatinin shown indicates that there was no reduction in the endogenous metabolism as the result of this treatment, but the amount of total nitrogen shows clearly that there was a very great change in the amount of protein material absorbed during the twenty-four hours. Singularly enough, the absolute quantity of ammonia on the day of catharsis was the same as the day before; but the percentage amount was enormously increased on account of the low excretion of total nitrogen. The catharsis in this patient must have been unusually severe (note the loss in weight), but there was no visible contamination of the urine from the bowel. The same phenomenon is to be seen in Case 3 on the day of catharsis, the rise in percentage being, however, very small as compared with Case 2, doubtless on account of a less complete purging. The absolute amount of ammonia was the same as the day before; and, by a coincidence, the same as for the corresponding day for Case 2. The rise in percentage for this day, while not more than might occur independently of such a cause, seems to be best explained by the fall in the total nitrogen. This emphasizes again the importance of great care in the interpretation of high ammonias.

## CREATININ NITROGEN

The chemical history of this fraction of the nitrogenous waste from the body has not yet been fully revealed. It seems to be proportional in amount to the muscular development of the individual<sup>22</sup> and therefore to be associated in some way with the functioning of this tissue, but not with the amount of work it does.<sup>23</sup> It has been strenuously held by some that the creatinin of the urine bears no physiological relationship with the creatin which is always found in the watery extract of muscle and which is chemically so closely related to creatinin.<sup>24</sup> But the recent experiments of Towles and Voegtlin<sup>25</sup> on the subcutaneous administration of creatin to fasting dogs and to dogs with the Eck fistula and the observation of Myers and Fine<sup>26</sup> that the daily output of the dog, cat

22. Shaffer: *Am. Jour. Physiol.*, 1908, xxiii, 1.

23. Shaffer: *Idem.*, xxii, 445; Von Hoogenhuyze and Verploegh, *Ztschr. f. physiol. Chemie*, 1905, xlv, 415; Pekelharing and Von Hoogenhuyze, *Idem.*, 1910, lxiv 262.

24. Folin: Hammersten's *Festschrift* 1906, p. 1; Von Hoogenhuyze and Verploegh, *Ztschr. f. physiol. Chemie*, 1908, lvii, 161.

25. Towles and Voegtlin: *Jour. Biol. Chem.*, 1912, x, 479.

26. Myers and Fine: *Jour. Biol. Chem.*, 1913, xiv, 9.

and rabbit is roughly proportional to the percentage content of creatin in their muscles, go far toward proving a close relationship between them. Until this point is settled we shall not be able to appreciate the exact significance of creatinin; but it will be interesting at all events to see how its elimination in the pregnant woman compares with that in normal non-pregnant individuals.

*Creatinin Coefficient.*—The number of milligrams of creatinin nitrogen excreted in twenty-four hours, per milligram of body weight, is known, after Shaffer<sup>22, 23</sup> (loc. cit.) as the "creatinin coefficient." Shaffer's average value for "37 supposedly normal individuals" was 8.1, maximum 11.7, and minimum 5.4. One of Shaffer's "pathological cases" was a woman in normal pregnancy. She was of "only fair muscular development, slender and active." Her creatinin coefficient in the seventh month of her pregnancy was 6.35, early in the ninth month it was 6.75, and one week before labor was 6.2—all obtained on a creatin and creatinin-free diet. Shaffer does not state whether he regards this as a low coefficient, but does accede to the statement of Benedict and Meyers<sup>27</sup> based on their study of the creatinin coefficient in insane but otherwise normal women that "the creatinin coefficient of women is, in general, lower than for men." Shaffer explains this as due to the relatively large amount of fat and smaller amount of muscular tissue in the average woman and expresses the belief that "sex *per se* has no significance." So far as we are aware no other coefficients for normal or non-pregnant women have been published. Neither Van Hoogenhuyze and ten Doeschate<sup>28</sup> nor Heynemann,<sup>29</sup> who have reported extensive studies in pregnant women, give the weights of their subjects.

From the results published by one of us<sup>5</sup> a short time ago, it is possible to calculate the coefficient for one case who was kept on a creatin and creatinin-free food for several weeks preceding her labor. Taking the average of twenty-one days the excretion of creatinin nitrogen was 0.24 gm. The average net weight for this period was 69.2 kilograms, which gives a coefficient of 3.5—but little higher than that found by Benedict and Myers for a woman convalescent from typhoid (namely, 3.1).

The average coefficients for that portion of the period of observation when the subjects of the present series were on a creatin and creatinin-free food (eleven days) are: 4.7 for Case 1, 3.2 for Case 2 and 4.7 for Case 3. It is probable that all four of these cases carried more body fat

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27. Benedict and Myers: *Am. Jour. Physiol.*, 1907, xviii, 377.

28. Von Hoogenhuyze and ten Doeschate: *Ann. de Gynéc.*, 1911, Series 2, VIII, 17 and 97.

29. Heynemann: *Ztschr. f. Geburtsh. u. Gynäk.*, 1912, lxxi, 110.



than Schaffer's "slender women," which no doubt explains in part the discrepancy between his coefficient and ours. Even his case, however, shows a lower coefficient than the average for a normal man, and, if sex *per se* plays no part, lower also than for the non-pregnant woman.

One reason for this will be apparent at once; namely, the dilution, if we may call it such, of the body weight by the uterine contents. Myers and Fine<sup>26</sup> have recently confirmed for the cat the observation originally made by Dörner<sup>30</sup> for rabbits, and later by Mellanby<sup>31</sup> for both rabbits and chickens, that young individuals have less creatin in their muscles than adults of the same species; and Rose<sup>32</sup> has found the creatin content of the muscles of the new-born infant to be only 0.07 per cent., as against 0.3 per cent. for the adult. Mendel and Leavenworth<sup>33</sup> have furnished us the only determinations yet made for the embryo, in the case of the pig, reporting .03 per cent. for an embryo of 265 mm. length as compared with 0.45 per cent., reported by Van Hoogenhuyze and Verploegh<sup>34</sup> for the muscles of the grown animal. Presumably the same relationship would hold for the human embryo of corresponding age, and the creatin content would gradually increase up to 0.07 per cent. found for the new-born infant.

We have been unable to find any analyses of the pregnant uterus for creatin. Judging by the analysis of the non-striated muscle of the stomach, bladder,<sup>35</sup> retractor muscle of the penis<sup>36</sup> and for the intermediate type of muscle in the heart,<sup>37</sup> it should be considerably less than that of striated muscle.

If creatin is the mother substance of creatinin in the urine, therefore, neither the fetus nor the uterus may be supposed to contribute much of it. The placenta, presumably, and the membranes and the fluids certainly, would contribute none at all. All of this weight would serve, as does the body fat, to reduce the creatinin coefficient. Even if creatinin does not come from creatin, we know from the fact that the elimination of creatinin by the new-born child is very small, that the production of this substance by the fetus must be negligible.

Support for this view is found also in the following observations, some of them unpublished, on a pregnant dog.<sup>12</sup>

30. Dörner: Ztschr. f. physiol. Chem., 1907, lii, 264.

31. Mellanby: Jour. Physiol., 1908, xxxvi, 447.

32. Rose: Jour. Biol. Chem., 1911, x, 265.

33. Mendel and Leavenworth: Am. Jour. Physiol., 1908, xxi, 100.

34. Von Hoogenhuyze and Verploegh: Ztschr. f. physiol. Chemie, 1905, xlvi, 432.

35. Saiki: Jour. Biol. Chem., 1908, iv, 483.

36. Buglia and Constantino: Ztschr. f. physiol. Chemie, 1912, lxxxi, 122.

37. Fürth, V., and Schwarz: Biochem. Ztschr., 1911, xxx, 413.

ON CREATIN AND CREATININ FREE FOOD			
Week of Gestation.	Weight, kg.	Creatinin per Twenty-Four Hours,	
		mg.	mg. per kg.
III .....	13.7	118	8.6
VII .....	15.2	118	7.7

ON DIET CONTAINING 275 GM. BEEFHEART			
Week of Gestation.	Weight, kg.	Creatinin per Twenty-Four Hours,	
		mg.	mg. per kg.
IV .....	13.9	135	9.7
IV .....	16.5	135	8.2
Third day after parturition ....	13.9	147	10.6

The return of the weight to 13.9 kg. so soon after delivery of the pups shows that the increase in weight in pregnancy was due mainly, if not entirely, to the product of conception and not to body fat. The rise of the coefficient after parturition is due to the discharge of some creatin from the involuting uterus in the form of creatinin.

This explanation may not be wholly adequate for the human organism. It will be impossible to determine whether it is or not until a rigid comparison is made between pregnant and non-pregnant women, whose weights are known, on proper diets.

Our observations on these cases having terminated nearly three weeks previous to delivery, the rise in creatinin excretion which was previously reported<sup>5</sup> as occurring in the last week or ten days of pregnancy does not appear here.

The creatinin and creatin metabolism just before and just after parturition has been fully discussed in former publications. Heynemann's work within the year has confirmed the conclusions we had already reached as to most of the points in discussion, and particularly as to the bearing which any alterations in this metabolism in normal pregnancy might have on the question of liver inefficiency. He argues, as did Murlin, that if it were shown satisfactorily that the liver does produce creatinin from creatin, and if it were shown further that in pregnancy the increase in creatin output is accompanied by a decrease in creatinin output, we might feel justified in accepting this as a sign of liver inefficiency, as do Van Hoogenhuyze and ten Doeschate. The former proposition, however, seems to be growing less rather than more secure as evidence accumulates, and on the latter there is no evidence at all as yet; for the tables of Van Hoogenhuyze and ten Doeschate do not show a decrease in the creatinin any more than do those of Heynemann or Murlin, and if they had followed the excretion of creatinin on a constant diet up to the day of parturition it is probable that they also would have seen a rise in this constituent rather than a fall. Heynemann's figures for the creatinin output vary so much from day to day that it is impossible to say whether his results confirm or refute his argument.

We are not inclined to lay much stress on the results from Eck fistula, especially if the hepatic artery be not tied; for Fischler<sup>38</sup> has shown that neither the formation of bile nor the carbohydrate tolerance is affected by this operation. Nevertheless, the newer experiments of Towles and Voegtlin<sup>25</sup> seem to show quite conclusively that whatever damage is done to the metabolic processes by this operation the transformation of creatin to creatinin is not one which suffers. They conclude that the liver is not an organ of prime importance in connection with the metabolism of these substances.

#### CREATIN NITROGEN

There is general agreement among the observations of Van Hoogenhuyze and ten Doeschate, Heynemann and ourselves as to the appearance of creatin in the urine of late pregnancy, even on a creatin and creatinin-free food. The three cases of the present series fall into line with previous ones. Case 2, however, shows less creatin than the others, and we can find no other explanation than the fact that the patient took more food per kilogram of body weight than the others. The appearance of creatin on the ninth and tenth days of the period may be the result of thyroid feeding on the eighth and ninth days. All the patients received on these days 15 grains of dried thyroid extract. This was the only case which showed any effect, and the only effect which any case showed.

It is possible that by feeding a larger amount of carbohydrate the urine could have been kept clear of creatin throughout. Mendel and Rose<sup>39</sup> have shown that for the rabbit at least a small amount of carbohydrate is sufficient to prevent or to diminish greatly the excretion of this constituent in the urine of otherwise fasting animals. As suggested in a former paper,<sup>5</sup> it is possible that the appearance of creatin in the urine of late pregnancy is due to the rapid diffusion of dextrose, the carbohydrate of the blood, through the placenta and its fixation or combustion there, thus depriving the maternal organism of its protecting influence. On December 16, the twelfth day of the experiment period and the day following thorough cleansing of the bowel, we fed two of the three patients (Cases 2 and 3) each 1 gm. of pure creatin,<sup>40</sup> the object being to test the capacity of the pregnant organism to eliminate or to utilize this form of nitrogen. Out of the 320 mg. of nitrogen administered, we recovered only about 60 mg. from Case 3, and none at all from Case 2 (a small quantity of urine was lost, however, from Case 3). Our hope was that by thoroughly cleansing the bowel and thereby reducing the

38. Fischler, cited by Wolf: *Jour. of Biol. Chem.*, 1912, x, 473.

39. Mendel and Rose: *Jour. Biol. Chem.*, 1911, x, 213.

40. The creatin was prepared for us by Dr. Stanley R. Benedict who also suggested the catharsis as a preparation for its administration.

possibility of bacterial decomposition a larger amount of the creatin fed might pass into the urine. The experiment is not very conclusive, but is in accordance with the previous results of Folin and Van Hoogenhuyze and Verploegh to the effect that creatin in the food is largely destroyed or is retained in the body of normal persons.

#### TOTAL PURIN NITROGEN

The total purin nitrogen excreted includes not only the nitrogen of uric acid, but also that of the less completely oxidized purin bases, hypoxanthin, xanthin, adenin and guanin, and that of any methylated compounds of this series like caffenin, theobromin, etc.

Excluding coffee, tea, etc., which might contribute methylated compounds to the urine, the amount of total purin nitrogen excreted on a purin-free diet is the best measure we have of nuclein metabolism in the body, i. e., of the destruction of cell nuclei. Following the terminology of Burian and Schur,<sup>41</sup> this is known as endogenous nuclein metabolism.

There is still a difference of opinion whether the uric acid should be regarded as an end stage in the metabolism of nucleins<sup>42</sup> or as only an intermediary stage on the way, by uricolysis and oxidation, to urea.<sup>43</sup> Concerning the other purins there is no doubt that they could be oxidized to uric acid if opportunity offered, so that the amount in which they appear in the urine is more or less fortuitous.

The amount of purin nitrogen excreted by the pregnant subject is a matter of some interest because of the important part which nuclei play in embryogenesis and histogenesis.

Our normal subjects were kept for eleven days on a diet which was nearly, if not quite, purin-free. Tea, coffee and cocoa were excluded. The output of total purin nitrogen, then, for these eleven days is a measure—a rough one probably—of the extent to which cells are being broken down and their nucleins destroyed. The tables give the average elimination for the entire fourteen days. When meat was ingested on the first two and last days, considerably more purin was found in the urine. Taking the average of the purin-free days we find that Case 1 eliminated on the average 0.36 gm., Case 2, 0.24 gm. and Case 3, 0.31 gm. purin nitrogen; or, expressed as a coefficient in milligrams per kilogram of body weight, Case 1, 4.8; Case 2, 3.7, and Case 3, 3.7.

The only experiments which we have been able to find on purin-free diets in which the total purin nitrogen was determined by a reliable method, are those of Cathcart<sup>44</sup> (Camerer-Arnstein method) on the

41. Burian and Schur: *Pflüger's Arch. f. path. Anat.*, 1901, lxxxvii, 239.

42. Wiechowski: *Arch. f. exper. Path.*, 1909, lx, 185.

43. Cf. e.g. Frank and Schittenhelm: *Ztschr. f. physiol. Chemie*, 1909, lxi, 269.

44. Cathcart: *Biochem. Ztschr.*, 1907, vi, 109.



professional faster Beauté, and those of Mendel and Lyman<sup>45</sup> (method of Krüger and Schmid) on normal male subjects.

Beauté, on an egg and milk diet, excreted 0.194 gm. of total purin nitrogen just before a fast of fourteen days, and 0.166 gm. on the same diet just after the fast. In milligrams per kilogram of body weight the coefficient was 3.0 and 2.8, respectively.

In Mendel and Lyman's experiments their subject, W. H. H., excreted 0.139 gm. total purin nitrogen on a purin-free diet, and J. F. L. excreted 0.144 gm. The coefficients per kilogram were 2.4 and 2.1, respectively.

It appears that the pregnant woman excretes not a little more purin nitrogen per unit of weight than does the normal man. It has long been known that the new-born infant excretes much more uric acid per unit of weight than does the adult, and more also in the relation to the urea excretion. Both Flensburg and Rensing<sup>46</sup> find the explanation of this fact in the hyperleukocytosis and the consequent high destruction of leukocytes going on in the infant's body during the early days of extra-uterine life. This hyperleukocytosis occurs also in the fetus and it is known that the leukocytes are destroyed both in the spleen and in the connective tissues.<sup>47</sup> Hence we should expect a rapid production of both uric acid and of purins which must be eliminated through the maternal system, since Mendel and Leavenworth<sup>48</sup> found no uricolytic enzyme in the embryo pig.

#### AMINO-ACID NITROGEN

Following the caution of Henriques and Sørensen, we wish to emphasize the fact that the fraction of nitrogen actually determined by the titration method includes some nitrogen in the form of polypeptids or other combinations; nevertheless it is customary to include all such free-acid groupings as can be set free for titration by neutral formaldehyd solution, after removal of ammonia, under this designation, and the method of Henriques and Sørensen certainly gives us as close an approximation to the free amino-acids as any that we have at present.<sup>49</sup>

Recent determinations of the amino-acid nitrogen in pregnancy by Falk and Hessky<sup>15</sup> and by Landsberg<sup>13</sup> working by the so-called "new method," show that this fraction is slightly increased over the amount found in the urine of the non-pregnant woman on a similar diet. The former observers found the average from non-pregnant subjects to be 0.17 to 0.29 gm. in twenty-four hours, or 1.9 to 2.8 per cent. of the total

45. Mendel and Lyman: *Jour. Biol. Chem.*, 1910, viii, 115.

46. Quoted by Czerny and Keller: *Ernährung des Kindes*, 1906, i, 216.

47. Minot: In Keibel and Mall's *Embryology*, 1911, ii, 504.

48. Mendel and Leavenworth: *Am. Jour. Physiol.*, 1907, xx, 97.

49. A comparison of the gasometric method of Van Slyke with the titration method of Henriques and Sørensen has been made on a variety of urines by Levene and Van Slyke. *Jour. Biol. Chem.*, 1912, xii, 309.

nitrogen. Landsberg reports 0.4 to 0.48 gm., or 2.5 to 3.0 per cent., of the total nitrogen. These figures agree fairly well with the percentage given by Henriques (2.2 per cent.) and with that by Levene and by Van Slyke<sup>49</sup> for normal urine.

In normal pregnancy Falk and Hessky find from 2 to 8 per cent. of the total nitrogen may be present in the form of amino-acids and Landsberg makes the figures from 0.23 to 0.67 gm., or 2.4 to 4.9 per cent. of the total. It will be observed that the absolute amount ranges even lower in the pregnancy urine than in that from non-pregnant women. Landsberg himself is disinclined to lay much stress on the very small increase which some cases show, and correctly refers a portion of the relative increase to the lower total nitrogen.

It will be seen from Tables 3, 4 and 5 that our results also show but a small increase over the amount considered normal. With Case 1 we have as the average of thirteen days, 0.47 gm. amino-acid nitrogen, or 4.4 per cent. of the total; with Case 2, for fourteen days, 0.26, or 5 per cent. of the total; and with Case 3 for thirteen days, 0.31 gm., or 3.3 per cent. of the total. All of these come within the range given by Landsberg. We call special attention to the fact that the lowest average absolute amount (0.26 gm.) is at the same time, owing to the low output of total nitrogen, the highest relative or percentage amount (5 per cent.). This shows how much the nitrogen retention may have to do with the percentage.

The agreement among all observers using a reliable method therefore is very close, and the lower figures should supercede those of Van Leersum,<sup>50</sup> who used his own modification of the old Schöndorff-Pfaundler method, and those of other observers who have denominated the rest nitrogen, after determination of urea, ammonia, creatinin and uric acid, as the "amino-acid nitrogen."

Falk and Hessky consider that their figures show a sufficient increase to indicate a distinct disturbance of metabolism and place their results on the side of those who regard the liver as the seat of this functional disturbance. In refutation of this view it is sufficient at this time to cite the new work of Folin<sup>51</sup> and his pupils and that of Van Slyke and Meyer,<sup>52</sup> which appear to demonstrate conclusively that neither the intestine nor the liver has any more to do with deamination of absorbed proteins than have other tissues, if they have as much. *High amino-acid nitrogen, therefore, can no longer be cited as proof of defective deamination in the liver.*

50. Van Leersum: Biochem. Ztschr., 1908, xi, 121.

51. Folin and Denis: Jour. Biol. Chem., 1912, xii, 87 and 141.

52. Van Slyke and Meyer: Jour. Biol. Chem., 1912, xii, 399.

It is only where an absolute increase of one- or two-tenths of a gram of amino-acid nitrogen or a relative increase of more than 2 per cent. can be shown, that any explanation, other than that of individual variation, analytical error or redistribution caused by retention of urea nitrogen, is called for. While we have no non-pregnancy cases for direct comparison, we doubt very much whether our results show any further departure than this. If, however, we accept the slightly higher results of Falk and Hessky we believe that we are bound to search for the explanation in some physiological peculiarity of pregnancy before we declare the result a pathological one.

In attempting to account for the condition of minus nitrogen balance which occurs in the dog at about the time corresponding to the period of morning sickness in the woman, one of us,<sup>53</sup> three years ago, offered the hypothesis that this period "simply marks the culmination of the more or less indiscriminate action of enzymes produced by the fetus" (fetal placenta) before the maternal placenta had been elaborated for the purpose of limiting the enzymes. Bar<sup>54</sup> had suggested a similar explanation (*une véritable mobilization des albumins*) for the loss of nitrogen, but did not commit himself as to the part played by the placenta.

Everything that we know about the implantation of the ovum certifies to its capacity to digest by proteolytic enzymes the uterine mucosa, and the morphological pictures which are presented by the process of vilus formation<sup>55</sup> bear witness that the trophoblastic cells which become the syncytial layer on the villus retain this capacity for a considerable time.<sup>56</sup> The demonstration by Graefenburg<sup>57</sup> of such enzymes in the human placenta, especially in the early months of pregnancy, and the recent discovery of Abderhalden<sup>58</sup> of enzymes in the blood of the pregnant woman, dog, cow and other animals, capable of splitting placental proteins, constitute significant support for the general view. Williams and Pearce<sup>59</sup> have found that these enzymes occurring in the blood of the pregnant woman are not specific, but are capable of acting on other proteins as well, and it is our belief that their presence in the blood is, as Abderhalden<sup>60</sup> admits to be possible, "simply a consequence of the commerce going on between mother and fetus, and signify only that a

53. Murlin: Am. Jour. Physiol., 1910, xxvii, 177.

54. Bar: Leçons en pathologie obstetricale, Paris, 1907, ii, 288.

55. Bruce and Teacher: Contributions to the Study of the Early Development and Imbedding of the Human Ovum; Glasgow, 1908.

56. Marshall: Physiology of Reproduction, New York, 1910, p. 484.

57. Graefenburg: Ztschr. f. Geburtsh. u. Gynäk., 1910, lxxv, 1.

58. Abderhalden and Kiutsi: Ztschr. f. physiol. Chemie, 1912, lxxxvii, 4.

59. Williams, P. F., and Pearce, R. M.: Proc. Soc. f. Exper. Biol. and Med., 1913, x, 73.

60. Abderhalden: Ztschr. f. physiol. Chem., 1912, lxxxi, 96.

very extensive transformation must take place before the material of the maternal blood can be carried over to the fetal blood." The action of placental enzymes has been offered by one of us also as an explanation of the higher amino-acid content of the urine of pregnancy.<sup>61</sup> If products of proteolysis going on in the placenta were diffusible into the maternal blood some of these products would pass to the kidney before there was an opportunity of their being deaminated or stored in the maternal tissues. The amount passing out by the kidney would not depend in any special way on liver function, but on the quantitative relation between their escape from the placenta and their destruction or fixation in the maternal tissues.

#### UNDETERMINED NITROGEN

Adding together all these fractions — urea, ammonia, creatin, creatinin, total purin and mono-amino-acid nitrogens — there yet remains a small fraction undetermined. Of course, we must recognize the fact frankly that what remains may be error. If an error of only 0.5 per cent. should occur in each of the determinations made and all of the errors were by chance in the same direction, there might be an apparent undetermined nitrogen of over 3 per cent. Assuming, however, that in most instances such errors would counterbalance each other, we find left in almost all of the urines we have examined an undetermined fraction of from 3 to 9 per cent. A very small part is in some cases due to albumin; in a few urines we have determined what Henriques and Sørensen call the polypeptid nitrogen; i. e., extra amino-acid nitrogen after digestion of the urine with strong hydrochloric acid. The indications are that this would account for a considerable part of what we have called in our tables the "undetermined nitrogen." Henriques and Sørensen have found such a fraction in normal urines. Falk and Hessky<sup>15</sup> have determined the polypeptid nitrogen in the urine of both pregnant and non-pregnant women and have found an increase of from two- to three-fold the normal amount in the former. Their percentage figures show about the same range as our percentage figures for the undetermined nitrogen. They believe the increase is due to an extra production of glycocoll in combination with aromatic and hydro-aromatic compounds similar to hippuric acid. We have no data from non-pregnant subjects with which to compare our results for this fraction. Granting, however, that it is increased, it seems to us more probable that compounds of the nature of oxyproteic acid, which Salomon and Saxe<sup>62</sup> have demonstrated to be present in the urines of normal preg-

61. Murlin: *Am. Jour. Physiol.*, 1911, xxviii, 450.

62. Salomon and Saxe: Cited by Falk and Hessky. See note 15.



nancy in larger quantity, or the non-dialysable substances which Savaré<sup>63</sup> found to be increased in such urines, are responsible.

The fact that the polypeptid nitrogen falls within twenty-four hours after delivery, as shown by Falk and Hessky, we should interpret to signify that it has its source in some function or process which ceases ordinarily with complete delivery of the child and placenta. This fact alone we should think would be sufficient to absolve the liver from responsibility quite aside from the new evidence that this organ has no special function to perform in relation to amino-acids or polypeptids; for it is difficult to see how it could recover its function completely in twenty-four hours. Still less could it be supposed to recover within this time if autolytic processes or degeneration of any other type within the liver parenchyma were the determining cause. Falk and Hessky were unable to find any polypeptid nitrogen in the press-juice of a fresh placenta and concluded from this that the placenta could not be the source of such bodies in the urine. Forty per cent. of the total nitrogen in this press-juice was in the form of amino-acid. This is an important fact in connection with the source of amino-acid nitrogen of the urine. Suppose, however, the amino-acids were to escape from the placenta together with proteolytic enzymes, some of which have been proved to be reversible in their action, i. e., can build up polypeptid compounds as well as split them into their component amino-acids. Might not synthetic compounds be formed either in the circulation or in the kidney which would pass through into the urine? The sudden decline of this fraction after parturition, at all events, would seem to be better explained by a fetal (placental) origin than by a maternal origin.

#### SUMMARY AND CONCLUSIONS

1. In a perfectly normal pregnancy and puerperium the percentage of ammonia nitrogen in the urine before and after labor shows but slight differences and lies within normal limits (4 to 6 per cent.), except for one or two days immediately following delivery, when it is slightly increased (7 to 10 per cent.).

2. Further evidence is presented that high ammonias may be encountered in normal cases due to contamination of the bladder. Irrigation of the bladder twice in twenty-four hours with saturated solution of boric acid caused reductions from 2.27 gm. to 1.33 gm. and from 1.07 gm. to 0.68 gm. ammonia nitrogen in twenty-four hours, the food and total nitrogen being the same.

3. Complete nitrogen partitions for three normal cases over a period of fourteen days in the ninth month of pregnancy are given. From these

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63. Savaré: Hoffmeister's Beitr. z. chem. Phys. u. Path., 1907, ix, 401.

results and from those of other cases previously reported, we deduce the following:

4. The percentage of total nitrogen eliminated as *urea* by a pregnant woman in the last month is somewhat lower than from a non-pregnant person, due largely to the retention of nitrogen for growth of the product of conception. The average for our three cases for fourteen days is 76.6 per cent. The range for six cases is from 70.8 per cent. to 79.4 per cent.

5. The *ammonia nitrogen* in perfectly normal pregnancies, in which the retention of nitrogen is large and the total nitrogen in the urine consequently small, may range as high as 12 per cent. of the total and be unaccompanied by any unfavorable symptoms. Immediately after severe catharsis it may reach the height of 17 per cent., because of the diminished absorption of nitrogenous food from the alimentary tract, and consequently diminished excretion of total nitrogen. For this reason and because of the variations in the elimination of total nitrogen due to variations in the diet, it is inadvisable to rely on percentage figures in the determination of the ammonia, a point long ago insisted on by F. Müller. The absolute values are much more reliable. In our experiments the absolute amount varies but slightly from day to day, and previous to delivery never exceeded 0.88 gm. in urine known to be uncontaminated. From these and earlier studies we are disposed to look on any amount up to .012 gm. ammonia nitrogen per kilogram of body weight in the twenty-four-hour urine as well within normal limits.

6. The *creatinin coefficient* of normal pregnancy is much lower than the creatinin coefficient of normal male subjects and is probably lower than that of normal non-pregnant women. This is due in part to the "dilution" of the body mass in pregnancy with material (fetus, fluids, membranes, uterine muscle,<sup>64</sup> etc.) which contain little or no creatin.

7. The occurrence of *creatin* in the urine of normal pregnancy is confirmed in the present cases. Its appearance, however, seems to be dependent, to some extent at least, on the amount of food taken.

8. The *total purin nitrogen* is slightly higher in the urines of normal pregnancy than in the urines of normal male subjects on similar diets. An explanation is found in the same facts which account for the high elimination of uric acid by the new-born infant.

9. The *amino-acid nitrogen*, as determined by the improved formol titration method, is but slightly increased above the amount considered normal in the urines of non-pregnant subjects. This slight increase may

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64. According to E. Mellanby [Proc. Roy. Soc. (B) 1913, lxxxvi, 88], the uterine muscle contains no creatin at all, and that it cannot be the source of the increased output of creatin in urine postpartum Mellanby believes he has demonstrated by collection of urines after hysterectomy.

be accounted for by supposing that proteolytic products, derived either from the placenta or from maternal tissues by action of placental enzymes, are carried by the circulation in larger amounts than usual to the kidney which is permeable to them.

10. The *undetermined nitrogen* is probably to be accounted for by polypeptid bodies, including oxyproteic acids.

11. In general, we believe that peculiarities in the composition of the urine of normal pregnancy as regards its nitrogenous constituents may be accounted for on purely physiological grounds.

# THE RENAL COMPLICATIONS OF HEMATIN INTOXICATION AND THEIR RELATION TO MALARIA \*

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Descriptions of the paroxysm<sup>1</sup> and of the changes in the blood<sup>2</sup> produced by the intravenous injection of alkaline hematin and their relation to human malaria have been presented in previous articles, and it is the object of this paper to add a third group of conditions which may properly be described as the renal complications of hematin intoxication.

The description of these renal complications is based on a study of two classes of material: First, the urine and kidneys of a large series of rabbits, in which the production of renal lesions and disturbances of function were not the objects of the experiments, but were merely incidental; and second, twenty rabbits, in which the production of renal complications was made the prime object of the experiments.

The hematin and hematin solutions used in these experiments were the same as in former experiments. The animals were fed, for the most part, on a variety of green foods, with hay and ground grains, and allowed to take water at liberty. The food in a given experiment was kept constant as to quality throughout the experiment, and the amount of water consumed during twenty-four hours was determined by supplying measured amounts in vessels so arranged that none could be wasted. The water remaining in the vessel at the end of twenty-four hours was measured and corrections for evaporation were made from control vessels. In only a few experiments were definite amounts of water given by stomach tube. The urine collected from metabolism cages was used for routine study, but the qualitative findings were confirmed by bladder urine in many instances. Observations were made on the normal urine for one or two days before the injection of hematin. In this connection it should be noted that the daily amount and character of rabbit's urine is so variable that but little importance can be attached to slight variations in the amount of urine, the urine-water ratio or the character of the urine. The results of the hematin experiments were controlled by a

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\* Submitted for publication April 21, 1913.

\* Aided by a grant from the Rockefeller Institute for Medical Research.

\* From the Pathological Laboratory of the University of North Carolina.

1. Brown, Wade H.: Jour. Exper. Med., 1912, xv, 579.

2. Brown, Wade H.: Jour. Exper. Med., 1913, xviii.



series of animals that received an equivalent volume of alkaline salt solution under the same conditions as those receiving hematin.

The extreme irregularity in the results of these experiments has shown conclusively that no given dose or combination of doses of hematin can be expected to produce a given effect on the kidneys of the rabbit, and the results reported are to be interpreted as the most probable effect from a given degree of hematin intoxication.

#### THE URINE

*Amount.*—A single dose of 10 mg. of hematin per kilo of body weight, or less, will produce no alteration in the daily amount of urine. If such doses are repeated from day to day, however, slight increases in the output of urine and in the ratio of the urine to the water consumed are gradually developed. The increased volume of urine is also present where slightly larger doses of hematin are administered, but as the dose approaches 20 mg. per kilo, there is a distinct decrease in both the daily amount of urine and the ratio of the urine to the water consumed. In exceptional instances there may be a complete suppression of urine for twenty-four hours or longer. On discontinuing the hematin, this phase of decreased output of urine gives place to increased urinary secretion and to an increase in the urine-water ratio, both of which exceed the normal within two to three days. This change is more enduring—in one animal it persisted for twenty-one days, when observations were discontinued.

*Color.*—The urine in hematin intoxication is highly colored, varying from a brownish-yellow to a dark reddish-brown and occasionally shows a greenish fluorescence. A smoky-red urine is seen in some instances.

*Acidity and Specific Gravity.*—The reaction and the specific gravity of the urine show no constant alterations. Both of these features vary widely in the rabbit under normal circumstances, and the variations after injections of hematin are therefore difficult to relate to any action of hematin.

*Albumin and Casts.*—A trace of albumin and a few hyalin or granular casts may appear in the urine after even a single injection of 10 mg. of hematin per kilo of body weight. The daily repetition of this dose of hematin seldom fails to produce an albuminuria with casts. As much as 20 mg. of hematin per kilo of body weight will produce a marked albuminuria with an abundance of hyalin, granular and epithelial casts, or even blood casts. Neither the amount of albumin nor the number or variety of casts, however, can be predicted from the amount of hematin injected.

*Blood and Hemoglobin.*—The most interesting phase of disturbed renal function, referable to hematin poisoning, is the occurrence of

hematuria and hemoglobinuria. Severe intoxication with hematin frequently results in hematuria of a variable degree. The amount of blood may be so slight as not to be suspected from the appearance of the urine, or so great as to give the urine a distinct red or smoky color. The condition is occasionally produced by a single large dose of hematin (20 to 25 mg. per kilo.); it occurs with greater frequency from the maintenance of a given concentration of hematin in the circulation for twelve to twenty-four hours. This can be done best by injecting three or four doses of 10 to 15 mg. of hematin per kilo within such a period of time. The test on the vitality of the animal is a severe one, and the size of the dose and frequency of repetition must be judged by the condition of the animal; even then many fatalities will result before the desired effect is produced.

Free hemoglobin in the urine, not associated with hematuria, has been observed in a very few instances. Hemoglobin was demonstrated by the guaiacum test in voided urine and bladder urine in five animals out of fifty examined. In two of these cases the guaiacum test was confirmed by the spectroscope. Three other animals gave a positive guaiacum test from voided urine which was not confirmed by bladder urine. Of these eight animals, all except three showed, post mortem, either hemorrhagic lesions of the kidneys, extensive hemorrhage into the peritoneal cavity, or both, which might have accounted for the presence of hemoglobin in the urine. Of the three undoubted instances of pure hemoglobinuria, one occurred in the course of a chronic intoxication, and the other two from the repeated injection of 20 to 25 mg. of hematin twice daily; one animal developed hemoglobinuria after the fourth injection, and the other after the seventh. All efforts to devise a method of administering the hematin that would produce this condition in a greater percentage of cases proved futile. Most of the attempts resulted in the production of hematuria. It is evident, therefore, that there are other factors than the hemoglobinuria that are essential to the production of a high concentration of hematin in the circulation.

#### THE KIDNEYS

Mild hematin intoxication, whether acute or chronic, produces no characteristic lesions in the kidneys. There is a slight increase in the size of the organs and a slight brown pigmentation. The epithelium of the convoluted tubules and of the ascending loops of Henle usually shows parenchymatous degeneration. In the chronic cases a few hyalin casts in the tubules and foci of round-cell infiltration in the boundary zone and in the cortex also furnish evidence of injury.

The injection of large doses of hematin, however, may produce very profound and characteristic lesions. In the acute stages the kidneys

are much enlarged and are uniformly dark-brown or purplish-red in color, or are diffusely mottled with small opaque yellow areas of necrosis and areas of hemorrhage. The kidneys are moist, the cortex is thickened and the straight vessels and glomeruli are intensely congested. The boundary zone usually shows the most pronounced congestion and irregular streaks of hemorrhage may extend from this zone into both the cortex and medulla. In rare instances, small infarcts are present. The most characteristic alterations are found in the glomeruli. The glomeruli are enlarged and their vessels are enormously dilated and congested. In some instances, many of the glomerular capillaries are occluded by hyalin masses that stain a brassy-red with eosin; such glomeruli are apt to show hemorrhage into the capsular space. On the other hand, the glomerular tuft may completely obliterate the capsular space. The glomerular epithelium is very slightly swollen and a few desquamated cells are present. To a less degree, other vessels of the kidney are dilated and congested, while many of the smaller vessels show hyalin thrombi or emboli. Greenish-brown pigment is found, especially in the glomerular capillaries, both as free granules and masses and within phagocytic cells.

The uriniferous tubules, more particularly the convoluted tubules and the ascending loops of Henle, show marked parenchymatous degeneration with desquamation of the epithelial cells, or even foci of necrosis. In extreme instances almost the entire cortical system may be necrotic. Occasionally, the tubular epithelium will show granules of greenish-brown pigment, much of which, as it reacts for iron only after oxidation with hydrogen peroxid<sup>3</sup> and is soluble in dilute alkalis, must be regarded as hematin. This pigmentation is more common in living than in necrotic cells. An abundant albuminous precipitate and many varieties of casts are found in the tubules throughout the kidney. Hemorrhage into the tubules is found in many cases of extreme intoxication. The hemorrhage is usually patchy in its distribution — seldom diffuse — and occurs in the region of those glomeruli that show hemorrhage or occlusion of their vessels. In some of these cases, there is also a diffuse staining of the epithelium with hemoglobin and granules or droplets of hemoglobin are found in the cells, in the lumen of the tubules and in the capsular space. The presence of hemoglobin was observed in three instances in which there was no hemorrhage. The presence of hemoglobin was verified by oxidation with hydrogen peroxid and obtaining an iron reaction, and by the presence of brownish, granular and crystalline deposits in the cells and lumen of the tubules in specimens fixed in a solution of formaldehyd immediately after the death of the animal.<sup>4</sup>

3. Brown, Wade H.: *Jour. Exper. Med.*, 1911. xiii, 477.

4. Browicz: *Virchows Arch. f. path. Anat.*, 1900. clxii, 373.

The interstitial tissues are usually edematous and there may be foci of hemorrhage. Foci of necrosis are usually surrounded and invaded by polymorphonuclear leukocytes.

In two instances anemic infarcts were observed from occlusion of relatively large vessels by masses of hyalin material.

In the early stages of recovery from hematin intoxication and at the time the kidneys are showing an increased urinary secretion, several important changes from the above description are to be noted. The glomerular tufts now decrease to normal size or are shrunken, while the capsular spaces and tubules are dilated and contain desquamated epithelial cells and an abundant albuminous precipitate.

In chronic intoxications, and as recovery from acute intoxications progresses, the kidneys are but slightly enlarged, but still show a brown pigmentation. The surface may present a few scattered areas of hemorrhage and necrosis, as in the acute stage, interspersed with small depressed scars. The glomeruli are irregular, some being enlarged while others are much contracted. The tubular epithelium shows various degrees of degeneration, with a slight desquamation, or even patches of necrosis. Many tubules show regenerating epithelium with numerous mitotic figures. In these areas of regenerating tubular epithelium there is usually a pronounced interstitial infiltration of plasma cells and an increase in the connective tissue.

#### DISCUSSION

Clinically and pathologically, slight degrees of hematin intoxication cause only such alterations in urine and kidneys as are commonly observed in many febrile conditions. In more severe intoxications the irregularity and uncertainty of the effect on the kidneys is quite striking: some of these cases again show only the disturbances of a febrile state, while others present the clinical picture of an acute nephritis and a very few a definite hemoglobinuria. Throughout, the one constant feature is the albuminuria with casts and degenerative lesions in the kidneys—all probably the result of a slight toxic injury. The wide range of variety in urine and kidney lesions that may be superimposed on this common basis under constant experimental conditions suggests strongly the element of chance in the effect of hematin on the kidneys. This chance factor is operative through the blood-vessels. It has been demonstrated by physiological methods that hematin produces a marked dilatation of the splanchnic vessels<sup>5</sup> and causes injury to the vessel wall. Microscopically, the glomerular vessels in particular show these changes and, in addition, obstruction to circulation in many areas by hyalin thrombi or

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5. Brown, Wade H., and Lovenhart, A. S.: Jour. Exper. Med., 1913, xviii. (In press.)



emboli. This occlusion of vessels seems to be the chance occurrence that determines the major part of the renal complications of hematin intoxication. The foci of tubular necrosis are but minute infarcts; the hemorrhage also is largely determined by vascular occlusion, although the injured and weakened vessel wall predisposes to hemorrhage.

A few kidneys have shown such a uniformity in the character and distribution of the lesions that one might be tempted to regard them as instances of acute nephritis, but in view of the great preponderance of lesions that are undoubtedly due to vascular occlusion and injury I am inclined to regard them all as of like nature.

No light has been thrown on the important subject of hemoglobinuria except that it is possible of production by intravenous injections of hematin in a very small percentage of cases. The hemoglobinemia resulting from hematin intoxication undoubtedly reaches the threshold value of a kidney showing vascular and tubular injury at a point below that of the normal kidney.

It is not possible to correlate closely the renal complications of hematin intoxication with those of human malaria. The disturbances of function and the lesions of the kidneys have been found much more pronounced in hematin intoxications than in comparable grades of malarial infection in man, while the predominance of the glomerular lesions observed in these experiments is not found in malaria. These differences are partly due to the difference in the concentration of hematin in the blood. It is believed, however, that the analogy is sufficiently close to render the facts disclosed by these experiments of value as a basis for a clearer comprehension of the mode of production of the renal complications of human malaria.

#### SUMMARY

1. Mild grades of hematin intoxication produce degenerative lesions in the kidneys and the urine shows a trace of albumin and casts.

2. Severe grades of hematin intoxication result in extensive dilatation, injury and occlusion of the renal vessels by hyalin thrombi or emboli, all of which are most pronounced in the glomerular vessels. Extensive degeneration and necrosis of tubular epithelium, hemorrhages and even anemic infarcts result from these vascular lesions. In such cases, the urine presents the characteristics of an acute nephritis.

3. In rare instances of severe hematin intoxication, hemoglobinuria may occur.

4. During the period of recovery from acute hematin poisoning and in chronic poisoning, the kidneys show both degenerative and proliferative processes. The glomerular tufts shrink and the capsular space and tubules are more widely dilated. The tubular epithelium shows degen-

eration and active regeneration with abundant mitotic figures. There are foci of round-cell infiltration and of connective tissue increase. There is also a slight diffuse increase in connective tissue. The urine is increased in amount and contains albumin with hyaline and granular casts.

5. The renal complications of hematin intoxication are believed to be due primarily to dilatation, injury and occlusion of renal vessels under the action of hematin.

## ON THE USE OF PITUITARY EXTRACT IN OBSTETRICS \*

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Although the first use of pituitary in obstetrical practice was made in England, but few papers dealing with its action have been published in English, while in Europe it has been so extensively used that a study of the large number of papers and cases now published enables one to formulate, with considerable precision, rules for its employment. This is especially true after one has given it, with success and failure, in one's own experience. This forms the justification for this paper.

In 1895, Oliver and Schaefer, in their series of experiments with organ extracts, followed up their papers on the striking effects produced by the injection of extracts of the adrenal by showing that extracts of the pituitary also produced a rise in blood-pressure. In 1898, Howell showed that it was the posterior lobe which possessed this property. This was confirmed by Schaefer and Vincent, and in 1901 Schaefer and Magnus showed that the extract of the infundibular portion increased very markedly the flow of urine. At this point the matter rested until 1906, when Dale, in the course of some observations on the action of ergot, noted that the extract of pituitary brought about a marked uterine contraction. This observation, however, was entirely lost sight of until Blair Bell and Hicks, led by some experiments which they had on hand, obtained from Dale some pituitary extract and produced with it marked contractions of the uterus in pregnant rabbits.

In consequence of the results obtained, Blair Bell was led to use it in some obstetrical cases. He presented his observations before the Liverpool Medical Institution, November, 1909, and his paper was published in December. In it he refers to its use in two cases of post-partum hemorrhage, and one case in which it was used in the expulsive stage of labor. In September, 1910, Aarons of Edinburgh read a paper at the International Congress in St. Petersburg, in which he reported success in its use in six cases of post-partum hemorrhage.

On June 25, 1909, Frankl-Hochwart and Fröhlich of Vienna reported on some experiments which they had made with pituitary extract. They were led to join forces in the study of the action of pituitary on the vegetative nervous system, as both of them had previous experience with

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\* From the Pharmacological Laboratory of the University of Toronto.

\* Submitted for publication July 2, 1913.

the pituitary gland under pathological conditions, and from what was known of the pharmacological action of the extract they were led to infer that it would produce uterine contractions. In this respect their hopes were answered, and they found that pregnant rabbits showed a very marked increase in uterine movement after the administration of pituitary. The effect on blood-pressure was by no means marked, although it was found to be considerable when injected into dogs. They also observed, as they had expected, very marked effects on the urinary bladder and were able to show that it not only stimulated, but increased the irritability of both the nerves to the bladder and to the uterus.

In conclusion, they suggested that it should be used in obstetrics, and it was not long before the advice was acted on. In January, 1910, Foges and Hofstätter tried it in some cases to arrest post-partum bleeding. They showed that it had little effect on blood-pressure, but quite promptly brought about uterine contractions. They had several failures, three at least in which no effect whatever was produced.

Early in 1911 Hofbauer published the first series of cases in which it was used to increase labor pains. In his twelve cases the effects were always exceedingly striking, the movements coming on or increasing in force within a few minutes. He saw in no case any indication of tetanus uteri, but in several cases regular storms of contractions, bringing on a very rapid delivery. The publication of this exceedingly optimistic paper has led to pituitary being very widely used, and at least seventy-five papers dealing with the use of pituitary in labor have been published. An examination of the literature has disclosed a total of at least 1,650 cases in which it has been administered. A complete list of references is appended. The author has avoided, as far as possible, counting any case twice. The latest paper and the highest number of cases by any author being taken to include those previously reported.

My attention was attracted to the advantages of pituitary extract in obstetrics from the results seen in animal experimentation in the pharmacological laboratory. In the course of researches which have been in progress in this laboratory, and for demonstrations for student classes, pituitary has been used a considerable number of times to increase uterine movements. The accompanying curve (Fig. 1) is shown as very typical. As may be seen from this tracing, the uterine movements have been very markedly increased in both rate and amplitude, while the tone, as judged by the elevation of the line joining the bases of the waves, has not been raised, save at first where an irregular tetanus may be seen. In none of the cases in which it was employed has a true tetanus uteri, i. e., a very marked rise in tone with comparatively slight superimposed movements, been seen. Hahl and Malinowsky have recorded the variations in intra-uterine pressure with a bag, after the method of Westermarck, and in





Figure 1



Figure 2

two cases reported by Hahl; there was some rise in tone, the movements being shorter and stronger, at shorter intervals and with increased intra-uterine pressure; and Malinowsky shows a good tracing of a uterine tetanus. In other cases no increase in tone occurred, but only of movements. Such tetani are, however, not uncommon with ergot preparations, but apparently much less so with pituitary. Cases have, however, occurred. In one of the cases published by Rieck a tetanus evidently occurred, and is very well described by him. When it took place after the second injection, which was given six hours after the first injection, the patient stated that she felt the individual pains, but the examining hand could detect no contractions or relaxations.

Lieven also reports a case in which the uterine tetanus was so marked that the child's heart-rate fell steadily to 82, and there was a marked passage of meconium. Spaeth reports a similar case. He was, however, so unfortunate as to see the child die. Seitz and Roemer have each stated that they have also seen tetanus occur. Hamm, too, in one case which was brought to him after the child was dead and the patient had been in labor for a considerable period of time, injected pituitary, and regular movements set in. These, however, died away, and a second injection was given, which was followed in seven minutes by a tetanic constriction which lasted eight minutes, then regular movements for one or two hours, which gradually died away. A third injection produced another tetanus of eleven minutes' duration, then regular movements; and the fourth, tetanus of seventeen minutes, followed by regular movements, which led to delivery. He states that he has frequently seen the fetal heart-rate fall to 80; but in view of the rapidity of delivery in most cases, does not consider this dangerous. The above incident shows quite clearly, however, that the use of pituitary is not without some danger to the child, though danger to the mother seems very slight. In consequence, the fetal heart sounds should be, if it is at all possible, kept constantly under observation. This danger seems undoubtedly greatest when the drug is administered during the first stage.

A study of the literature has disclosed that the majority of observers, and especially those with the larger series of cases, have found that for the production of abortion, pituitary alone is insufficient. We might quote in this connection Schiffmann, who, out of seven cases, had three complete failures. Hell, Fischer, Nagy, Hirsch, Voigt, Merkel, Sellheim and Trapl have all pronounced against its use for this purpose.

Nor has it been of any great service in initiating premature labor. Trapl, Schiffmann, Fischer, Nagy, Hirsch, Voigt, Foges and Hofstätter, Merkel and Sellheim may be quoted in support of this statement. Nevertheless, successful cases have been observed in which one or two injections sufficed; and in the production of both abortion and premature labor, it

has proved in almost all cases a useful aid to other methods, such as dilatation by mechanical means.

Practically all observers are unanimous in declaring that it is of the greatest value in overcoming uterine weakness supervening after dilatation of the soft parts, and during the expulsive stage of labor. The effect, as a rule, is very prompt. The pains set in with great vigor in fifteen to twenty minutes or less, and are strong, rapidly leading to delivery; sometimes in a few minutes, and frequently within the hour. As mentioned above, pituitary has been used in at least 1,650 cases, but it is difficult to estimate in how many of these cases it was given during this phase of labor. Not more than a dozen failures during this stage are recorded. The total number of complete failures reported is less than fifty, and these failures are largely amongst those cases in which it was used for production of abortion or premature labor, without other means being employed, or post partum. Several observers have found that it produces movements that are so rhythmical and strong as to be of the greatest value in converting abnormal into normal positions. There is, of course, no general agreement as to what constitutes failure; and as details in all the longer series of cases are not given, it is impossible to express the results recorded in the literature in a more exact fashion. Hofbauer, in his last forty cases, reports no failures; Cahn, out of eighty-seven cases, three; Aubert, 15 per cent. of failures; Foges and Hofstätter, out of sixty-three cases, three failures. These figures seem very typical; but it must be noted that other observers, with as large a series or larger, say nothing of failure.

Its action in these cases is well illustrated by the following cases, selected from amongst those seen by me.

CASE 1.—Primipara, 23 years of age. Labor pains began about 3 a. m. When examined at 9 a. m. the cervix was soft and easily dilatable; but the pains were feeble, and during the course of the pain there did not appear to be any advance of the head, which was in the first position. The patient was seen several times through the day; and the soft parts, including the vagina and perineum, became gradually softened; but still the pains were very feeble, did not advance the head and caused the patient no distress whatever. At 4:30 p. m. 1 c.c. of infundibular extract, prepared by Messrs. Burroughs, Wellcome & Co., was injected. Strong expulsive pains began in five minutes. The contractions were regular and continuous. The head descended quickly and the child was born at 4:45 p. m. The uterus was well contracted. The placenta separated and was expelled in five minutes. Without pituitary, one would undoubtedly have used forceps in this case, as the patient was becoming rather tired, and would apparently have gone on for hours without delivering herself.

CASE 2.—III-para, 25 years of age. This was the third pregnancy in three years, and she had not been feeling very strong during the latter weeks. Labor began at 9 p. m. The pains were only slight all night, but sufficient to dilate all the soft parts. They occurred every five minutes, but seemed to have no expulsive power. The patient did not appear to help herself very much, and as she was becoming tired, one had to consider interference. It was decided to try pituitary before forceps. One c.c. of the infundibular was injected at 7:20 a. m. In five

minutes strong rhythmical contractions occurred, which caused a rapid expulsion of the child in less than ten minutes. The placenta was expelled in five minutes.

Pfeifer, Sterne, Zinsser, Jaeger, Mory and Hamm have seen failure to produce delivery, owing to failure of the soft part to dilate. Hamm, indeed, saw physiological stricture develop in four cases where it was used to produce premature labor.

In the two cases of failure seen by me, the difficulty appeared to be that the soft parts did not become dilatable. No ill effects were produced, but forceps had to be applied.

CASE 3.—Primipara, 23 years of age. Labor began at 9 a. m. There were feeble pains all day, which became more severe at 10 p. m. The head was in the first position. Dilatation was progressive and the head descended. Then the pains became feeble, and had little expelling power. One c.c. of infundibular extract (B., W. & Co.) was injected. Stronger pains came on within twenty minutes, but the perineum did not relax, nor did the patient seem to help herself. Finally the forceps were applied and delivery effected. The uterus was well contracted, and the placenta was expelled within ten minutes.

CASE 4.—Primipara, 28 years of age. There were feeble pains throughout the day, which became more regular about 11 p. m. When seen at 2:30 a. m. the pains were occurring at about five-minute intervals. The patient was very nervous and did not help herself very much. The cervix was well dilated, and the pains were not very strong. One c.c. of infundibular extract was injected. In about twenty minutes the pains, as judged by the patient's sensations, became more severe; but the contractions were not appreciably quickened, nor did they advance the head, which was in the first position and fairly low down, although not bulging the perineum. After waiting some time, and as the patient was becoming tired, forceps were applied and delivery effected at 6 a. m. The uterus contracted well, and the placenta was expressed in ten minutes.

In this case the injection of pituitary extract did not seem to increase at all the expulsive power of the uterus; but there appeared to be a distinct lack of voluntary efforts on the part of the patient.

Several observers warn their colleagues not to employ pituitary after delivery of the child and before that of the placenta. Rieck reports a case of retention of the placenta. Hirsch, Voigt, Foges and Hofstätter, Rieck, Merkel, Seitz, Kroemer, have all found it not so valuable post partum; and several of these express a preference for ergot. In view of the greater frequency with which the latter in laboratory experience produces tetanus uteri, this is easily understood. Sellheim reports that out of twelve cases in which he used it, eleven were failures.

Several observers state that they have found it of no value in uterine atony; e. g., Andres; and this condition probably explains the cases of complete failure to reawaken pains when they have prematurely ceased, such as reported by Zinsser (five out of sixty-five cases), Hamm (one case in forty).

In addition to the action of pituitary on the uterus, its action in increasing the movements of the urinary bladder are important. Jaschke and Franz have employed it as a postoperative tonic for this purpose;



and several of those who have used it in obstetrics have noted that the bladder was well emptied without the use of a catheter.

That in pituitary we have a drug which will increase the flow of milk, was shown by Mackenzie and confirmed by Ott and Scott, and may be illustrated by the curve produced in Figure 2. In this experiment the method employed was that of Mackenzie. A lactating cat was anesthetized, the skin removed from over two mammae, their nipples cut off, and the gland deeply incised. An injection of pituitary produced the prompt response shown in the tracing. In all, some 5 c.c. of milk were secreted. After an interval of thirty minutes a further injection produced an increased flow. The effect of an intravenous injection is evidently very marked, but brief. Whether single or even daily injections will lead to a permanent increase in milk production is not decided. The cases advanced in support of this view by Reynolds are not sufficiently numerous, nor were the observations recorded with sufficient care to be convincing.

#### CONCLUSIONS

1. Pituitary is of great value in cases of weakness in uterine movements after the soft parts are well dilated. Failure in these cases is rare, probably less than 1 per cent. The later in labor, but before delivery, the more striking the effect. The danger to the child and mother is very slight.

2. As an addition to some mechanical method, e. g., the Champetier de Ribes' bag, it is of great value in bringing on premature labor or abortion. In the former case it may be sufficient in itself, but there is some risk of tetanus of the cervix, or of the uterus, especially when repeated injections are required.

3. For delivery of the placenta its use is accompanied by the danger of tetanus uteri and retention.

4. In post-partum hemorrhage a considerable percentage of failures may be expected.

When a need for a uterine stimulant arises in cases conforming to the above indications, I believe that pituitary is of the greatest value, and will act as in Cases 1 and 2, which are typical of others in my experience.

I desire to express thanks to Dr. V. E. Henderson for the use of his laboratory, and invaluable assistance and criticism in preparing this paper.

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# THE TREATMENT OF SYPHILITIC AFFECTIONS OF THE CENTRAL NERVOUS SYSTEM WITH ESPECIAL REFERENCE TO THE USE OF INTRA- SPINOUS INJECTIONS \*

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Syphilis of the central nervous system is a manifestation of an infectious disease caused by a known parasite, against which we have at least two specific therapeutic agents. It is, therefore, amenable to treatment. The problems to be solved are: (1) How to recognize the condition before irreparable damage has been done to important structures; (2) the determination of the most efficient methods of application of our curative agents. The first can be solved only by careful clinical study and routine examination of the cerebrospinal fluid of all suspected patients. It is the purpose of this communication to present the results of an attempt to solve the second problem, viz., the determination of the best method of application of the curative agents.

The demonstration of the *Treponema pallidum* in general paralysis has definitely established the fact that in the so-called parasyphilitic nervous diseases there is active syphilis of the central nervous system. The cerebrospinal fluid in paresis and tabes shows qualitatively the same alterations as does the fluid in cerebrospinal syphilis. The usual changes occurring in the cerebrospinal fluid in these conditions are pleocytosis, with a predominance of mononuclear forms, and an increase in globulin. These abnormalities indicate the presence of a chronic inflammatory process in the cerebrospinal axis. A positive Wassermann reaction in the fluid indicates that the inflammation is syphilitic in nature. These abnormal constituents usually disappear more rapidly under specific treatment in involvement of the nervous system occurring early, than they do in such lesions occurring late in syphilis or in tabes or general paralysis, especially the latter. In late syphilis the spirochetes have established themselves in strongholds difficult to reach with our drugs. Such strongholds are the regions of poor vascularization, and in these the

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\* From the Hospital of the Rockefeller Institute for Medical Research.

\* Read before the Association of American Physicians May 7, 1913.

\* Submitted for publication June 30, 1913.



central nervous system is particularly rich. The *Treponema pallidum* has a predilection for lymph spaces. For practical purposes the perivascular lymphatics and the perineuronal spaces may be considered as lymph spaces which are not in direct communication with the general lymphatic system, but which are part of the subarachnoid space. Through them flows the cerebrospinal fluid. It has been well established that there is very little excretion of curative agents from the blood into the cerebrospinal fluid. Flexner and his co-workers have shown the necessity of directly introducing the therapeutic sera into the subarachnoid space in the various forms of purulent meningitis. The same principle no doubt holds true, to a considerable degree, in all forms of meningitis.

In dealing with syphilis of the central nervous system, we are, however, more fortunate than is the case in purulent meningitis. For here the introduction of our therapeutic agents into the general circulation is of undoubted benefit. With mercury and iodids and with salvarsan intravenously much can be done, and in many patients all clinical signs and symptoms can be relieved. Efficient therapy, however, means more than the relief of symptoms; it involves also the eradication of all evidence of disease. This is more difficult. The changes occurring in the cerebrospinal fluid — pleocytosis, increased globulin and positive Wassermann reaction — must, as has been stated, be looked on as evidence of active syphilis of the central nervous system. No treatment can be considered adequate which does not result in the permanent disappearance of these abnormalities.

While rapid symptomatic improvement frequently follows the use of mercury and iodids, the relatively slight influence of these drugs on the abnormal constituents in the spinal fluid suggests at once the probability of subsequent relapse. In tabes the use of mercury seems frequently actually to increase the intensity of the symptoms. Salvarsan intravenously acts more rapidly than mercury, especially in reducing the degree of pleocytosis. To produce a disappearance of the increased globulin and to change the Wassermann reaction from positive to negative is, however, much more difficult.

The idea of intensifying the treatment of syphilis of the central nervous system by the introduction of salvarsan directly into the cerebrospinal fluid naturally suggests itself. To determine whether this might be safely done, we injected high dilutions of the drug in monkey-serum intraspinaly into monkeys. Subsequent examination of the cerebrospinal fluid showed that the drug introduced in this form was too irritating to warrant its application to patients. Neosalvarsan injected intraspinaly into monkeys was less irritating, but when injected into patients

produced symptoms too severe to warrant its continued use.<sup>1</sup> Serum, on the other hand, can be repeatedly injected into the subarachnoid space without demonstrable injury to the nervous tissue.

The blood-serum of patients treated intravenously with salvarsan has been shown to have definite therapeutic value when injected subcutaneously into patients with congenital and secondary syphilis.<sup>2</sup> We have been able to show that this serum also exerts a spirocheticidal action on the spirochetes of relapsing fever.<sup>3</sup> When the serum is allowed to act on the spirochetes *in vitro* and subsequently these are injected into mice, they do not develop in the mice as do untreated spirochetes or spirochetes on which normal serum has been allowed to act. Heating the serum to 56 C. increases the spirocheticidal action. We have also used the serum of syphilitic patients to make serum agar for the growth of *Treponema pallidum* after Noguchi's method. Cultures in media made with serum obtained before treatment grew practically as well as in that made with normal serum, while in media made with serum obtained an hour after intravenous injections of salvarsan the spirochetes grew more slowly or not at all. In media made with serum which was obtained at longer intervals after treatment, the growth approximated that in normal control tubes. These experiments indicate that the serum has the greatest inhibitory effect shortly after the injection of salvarsan.

All the above-mentioned experiments support the view that the serum of salvarsan-treated patients has a definite antispirochetal effect, both *in vitro* and *in vivo*. Such serums are ideal preparations for direct

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1. Wechselmann (Deutsch. med. Wchnschr., 1912, xxxviii, 1446), reports that he had injected neosalvarsan intraspinaly into two patients with general paralysis, and into two children with congenital lues, without any injurious effects. Marinesco (Ztschr. f. physik. u. diätet. Therap., 1913, xvii, 194), reports the results in thirteen patients, each of whom received 5 mg. neosalvarsan in 4 c.c. solution intraspinaly. In ten of these severe unpleasant symptoms immediately appeared. Eight of them had permanent bladder disturbance. From this experience Marinesco advises strongly against the intraspinal injection of neosalvarsan. We are in complete accord with his conclusions. In the same communication Marinesco states that he had treated patients with syphilis of the central nervous system with serum obtained from patients with secondary syphilis after treatment with salvarsan. There were some beneficial results, but the work was not continued.

Robertson (Edinburgh Med. Jour., 1913, x, 428), reports on the intraspinal treatment of patients with general paralysis. For this treatment he used the serum of patients with secondary syphilis, which was withdrawn on the third day after a salvarsan injection, and also the paretics' own serum withdrawn one hour after the intravenous injection of salvarsan. Only a few treatments were given to each patient, but half of those treated showed some improvement.

2. Meirowsky and Hartmann: Med. Klin., 1910, vi, 1572; Plant, H. C.: Deutsch. Med. Wchnschr., 1910, xxvi, 2237; Gibbs and Calthrop: British Med. Jour., 1911, i, 809.

3. To be published. Gonder, R. (Ztschr. f. Immunitätsforsch. Orig. 1912, xv, 257), reports on a similar spirocheticidal action of the blood of salvarsan-treated rats on the spirochetes of relapsing fever.

introduction into the subarachnoid space, and for the past two years we have been studying the results of the injection of such serums intraspinously into patients with syphilitic disease of the central nervous system.

#### TECHNIC OF SUBARACHNOID INJECTIONS

The technic that we have employed varies but little from that previously described,<sup>4</sup> and is as follows:

One hour after the intravenous injection of salvarsan 40 c.c. of blood is withdrawn directly into bottle-shaped centrifuge tubes, and allowed to coagulate, after which it is centrifugalized. The following day 12 c.c. of serum is pipetted off and diluted with 18 c.c. of normal saline. This 40 per cent. serum<sup>5</sup> is then heated at 56 C. for one-half hour. After lumbar puncture the cerebrospinal fluid is withdrawn until the pressure is reduced to 30 mm. cerebrospinal fluid pressure. The barrel of a 20 c.c. Luer syringe (which has a capacity of about 30 c.c.), is connected to the needle by means of a rubber tube about 40 cm. long. The tubing is then allowed to fill with cerebrospinal fluid so that no air will be injected. The serum is then poured into the syringe and allowed to flow slowly into the subarachnoid space by means of gravity. At times it is necessary to insert the plunger of the syringe to inject the last 5 c.c. of fluid. It is important that the larger part of the serum should be injected by gravity and if the rubber tubing is not more than 40 cm. long the pressure cannot be higher than 400 mm. Usually the serum flows in easily under even a lower pressure. By the gravity method the danger of suddenly increasing the intraspinal pressure to the danger point, such as might occur with rapid injection with a syringe, is avoided. Frequently there is a certain amount of pain in the legs, commencing a few hours after the injection. The pain is more often noticed in tabetics than in patients with cerebrospinal syphilis. It can usually be controlled by means of phenacetin and codein. Occasionally morphin is required.

The therapeutic effect of this form of treatment is illustrated in the tables, where the treatment, Wassermann reaction in the blood and in the cerebrospinal fluid, the cell count and globulin in the fluid of each patient are given. In some of the tables, the Wassermann reaction in the cerebrospinal fluid is indicated with two different antigens. Prior to January, 1913, fetal liver extract was used as antigen. It was then found that an alcoholic extract of human heart, to which 0.4 per cent. cholesterin had been added, was a much more sensitive reagent.<sup>6</sup> In order not to confuse the results it was considered wise to continue the use of the liver antigen in those cases in which it had previously been used, and to add the comparative reactions with the cholesterin-heart antigen. Unless otherwise designated in the notes after January, 1913, a completely negative reaction indicates that it was negative with the cholesterin-heart as well as with the liver antigen. In the cerebrospinal fluid column, the amount of fluid which gave complete fixation is indi-

4. Swift, H. F., and Ellis, A. W. M.: *New York Med. Jour.* 1912. xevi. 53.

5. In patients who do not have reactions following the injection of 40 per cent. serum the strength is at times increased to 50 or 60 per cent., or even stronger.

6. Walker, I. C., and Swift, H. F.: *Jour. Exper. Med.* 1913. xviii. 75.

cated.<sup>7</sup> The quantitative titration of the strength of reaction has been used since November, 1911. Previous to that time, only 0.1 c.c. of fluid was used. The titration of the strength of the reaction furnishes a fair index of the effect of treatment. In this report we wish to lay more emphasis on the improvement in the condition of the cerebrospinal fluid than on the clinical improvement, because it is entirely objective and probably gives us a better idea of the actual effect of our therapeutic measures.

TABLE 1.—No. 85. C. L. V. AGE 35. TABES DORSALIS SIX YEARS. SYPHILIS SIXTEEN YEARS. RESULT: IMPROVED

Date, 1910	Blood Wasser- man Reaction	Cerebrospinal Fluid			Treatment	
		Cells per c.mm.	Noguchi Globu- lin	Wassermann Reaction, c.c. Spinal Fluid	Intra- venous Salvarsan, gm.	Intraspinous Serum
Nov. 10 to. 1911	++	...	....	.....	‡	.....
Jan. 10 ...	+	...	....	.....	.....	.....
Jan. 16 ...	±	125	++	0.1 —	0.2	.....
Jan. 25 ...	+	...	....	.....	0.3	.....
Feb. 8 ...	+	...	....	.....	0.36	.....
Feb. 13 ...	....	150	±	0.1 —	.....	.....
Feb. 15 to..	±	...	....	.....	†	.....
Aug. 20 ...	....	...	....	.....	†	.....
Aug. 21 ...	±	63	+	0.1 ± ?	†	.....
Aug. 26 to..	....	...	....	.....	.....	.....
Sept. 22 ..	± ?	...	....	.....	§0.2	.....
Sept. 22 ..	....	26	±	0.1 —	.....	.....
Nov. 24 ...	....	...	....	.....	0.2	.....
Dec. 1* ...	± ?	50	+	0.5 ±	0.2	18 c.c. of 50%
Dec. 11 ...	±	20	+	.....	0.2	30 c.c. of 50%
Dec. 18 ...	±	20	±	0.5 ±	0.2	20 c.c. of 40%
1912						
Jan. 5 ....	—	12	±	0.5 ±±	0.3	30 c.c. of 40%
Jan. 12 ...	—	4	—	0.5 ±	0.3	30 c.c. of 50%
Jan. 15 ...	—	...	....	.....	0.2	.....
Jan. 20 ...	....	5	—	0.5 ± ?	.....	.....
July 9 ....	—	2	—	0.5 —	.....	.....
Sept. 10 ..	—	1	—	0.5 —	.....	.....
1913						
May 15 ..	—	3	±	0.5 —	.....	.....

\* Where both intravenous and intraspinous treatment were used the date of the intravenous injection is indicated. The intraspinous injection was given on the following day.

† Mixed treatment by mouth.

‡ Sixteen injections HgCl<sub>2</sub> intramuscularly.

§ Five injections given.

7. In performing the reaction with the cerebrospinal fluid 0.5 c.c., 0.4 c.c., 0.3 c.c., 0.2 c.c., 0.1 c.c. and 0.05 c.c. fluid are used. As we employ one-half quantities of all the reagents as compared with the amounts originally recommended for the Wassermann reaction, these quantities therefore correspond to 1.0 c.c., 0.8 c.c., 0.6 c.c., 0.4 c.c., 0.2 c.c. and 0.1 c.c., respectively.



## CASE REPORTS

CASE 85.—C. L. V., a well marked tabetic, who had had symptoms for about six years, lately becoming more marked. He had lightning pains, difficulty in walking, girdle sensation about the abdomen, and Charcot joint in the right hip. He had received practically no antisyphilitic treatment prior to November, 1910. In the first year of treatment he received sixteen intramuscular injections of mercuric chlorid, nine intravenous injections of salvarsan, a total of 2 gm., and six months' mixed treatment by mouth. The result of this was to reduce the pleocytosis from 125 to 50 per cm. The effect on the Wassermann reaction in the cerebrospinal fluid was not determined, because an insufficient quantity of fluid was employed. After the institution of intraspinal treatment with his own serum the fluid became normal in six weeks. During this period a total of 1.4 gm. salvarsan was given in six intravenous injections and five intra-spinal injections, a total of 60 c.c. of undiluted serum.<sup>6</sup> For over a year the fluid has

TABLE 2.—No. 213. O. W. A. AGE 29. TABES DORSALIS TWO AND ONE-HALF YEARS. SYPHILIS NINE YEARS. RESULT: IMPROVED

Date, 1911	Blood Wass. Reaction	Cerebrospinal Fluid				Treatment	
		Cells per c.mm	Noguchi Globulin	Wassermann Reaction		Intra- venous Salvar- san, gm.	Intraspinal Serum
				Liver Antigen, Spinal Fluid c.c.	Choles- terin Heart Antigen, Spinal Fluid, c.c.		
June 23 to	++	...	...	.....	.....	.....	.....
Aug. 2 ....	++	...	...	.....	.....	5 x 0.2	.....
Aug. 5 ....	....	110	—	0.1 ±	.....	.....	.....
Aug. 10 to	++	...	...	.....	.....	.....	.....
Oct. 20 ...	+	...	...	.....	.....	4 x 0.2	.....
Oct. 27 ...	+	75	—	0.1 ++	.....	0.2	25 c.c. of 40%
Nov. 3 ...	++	...	...	.....	.....	0.2	.....
Nov. 21 ...	++	...	...	0.1 ++	.....	0.2	20 c.c. of 50%
Dec. 12 ...	++	20	±	0.1 ++	.....	0.2	30 c.c. of 70%
Dec. 19 ...	++	19	±	0.2 ++	.....	0.2	25 c.c. of 40%
1912							
Jan. 5 ....	+	22	—	0.1 ++	.....	0.3	30 c.c. of 50%
Jan. 12 ...	+	8	—	0.3 ++	.....	0.3	30 c.c. of 50%
Jan. 19 ...	+	10	—	0.3 ++	.....	0.3	30 c.c. of 50%
Jan. 25 ...	+±	8	—	0.2 ++	.....	0.3	30 c.c. of 50%
Feb. 1 ....	....	5	—	.....	.....	0.3	25 c.c. of 40%
Feb. 8 ....	++	7	—	0.2 ++	.....	0.3	30 c.c. of 50%
April 11 ..	+	2	—	0.3 ++	.....	0.3	30 c.c. of 40%
April 18 ..	±	...	...	.....	.....	0.3	.....
April 25 ..	++	10	—	0.3 ++	.....	0.3	30 c.c. of 40%
May 2 ....	++	8	—	0.3 ++	.....	0.3	30 c.c. of 40%
May 16 ...	+	10	—	0.3 ++	.....	0.3	30 c.c. of 40%
1913							
Feb. 22 ...	++	1	—	0.4 ++	0.3 ++	*0.75	30 c.c. of 40%

\* Neosalvarsan.

6. In the tables the amount of diluted serum which was injected at each treatment is shown, while in the abstracts in the text the total amount of undiluted serum is given.

remained normal and the patient has been practically free from pain. The Charcot joint has prohibited his return to his usual work, although he goes about very well. There has been no advance in his tabes.

CASE 213.—O. W. A. had a rapidly progressing tabes of two and a half years' duration. The most marked symptoms were ataxia, pain and hyperesthesia in the lumbar region. In the first four months of treatment a total of 1.8 gm. of salvarsan was given in nine intravenous injections, which produced a drop in cells from 125 to 75, but had no effect on the Wasserman reaction in the cerebrospinal fluid. On the institution of intraspinal injection of patient's own serum there was an immediate drop in the number of cells. In the next seven months he received 4.3 gm. salvarsan in sixteen intravenous injections and fourteen intraspinal injections, a total of 184 c.c. undiluted serum. Under this treatment the cells were reduced to practically normal in number, and the Wassermann reaction in the cerebrospinal fluid was much reduced in strength. Because the patient felt so much better he neglected to report for nine months, during which time the fluid remained practically the same.

A marked beneficial effect was noted in both these patients when the intraspinal treatment was instituted, but the objection might be advanced that the improvement was due to the continuation of the salvarsan intravenously. To determine the effect of the intraspinal treatment alone, several patients were treated intraspinaly with the serum from other patients who had received intravenous injections of salvarsan.

TABLE 3.—No. 557. C. H. L. TABES DORSALIS TEN YEARS. SYPHILIS THIRTY YEARS. RESULT: IMPROVED

Date, 1911	Blood Wass. Reaction	Cerebrospinal Fluid			Treatment	
		Cells per c.mm.	Noguchi Globulin	Wassermann Reaction, c.c. Spinal Fluid	Intra- venous Salvar- san, gm.	Intraspinal Other Patients' Serum
Oct. 25....	—	42	++	0.1 ++	.....	.....
Oct. 30....	..	41	++	0.1 ++	.....	25 c.c. of 40%
Nov. 6....	..	40	++	0.1 ++	.....	20 c.c. of 40%
Nov. 18....	..	50	++	0.2 ++	.....	22 c.c. of 40%
Dec. 3....	—	18	++	0.2 ++	.....	26 c.c. of 50%
Dec. 12....	..	17	++	0.1 ++	.....	30 c.c. of 50%
Dec. 28....	..	15	++	0.4 ++	.....	25 c.c. of 40%
1912						
Jan. 6....	..	15	+	0.1 ++	.....	25 c.c. of 40%
Jan. 12....	..	10	++	0.2 ++	.....	30 c.c. of 50%
Jan. 23....	..	14	++	0.5 ±?	.....	26 c.c. of 50%
Jan. 31....	..	16	+	0.5 ±	.....	30 c.c. of 50%
Feb. 7....	..	17	+	0.5 +±	.....	30 c.c. of 50%
March 4....	—	15	+	0.5 +±	.....	.....
April 10....	..	10	±	0.5 ±	.....	.....
April 16 to	—	..	....	.....	.....	.....
May 14....	—	..	....	.....	*0.3	.....
May 14....	..	19	+	0.5 ±	.....	.....
Sept. 24....	—	8	±	0.5 —	.....	.....
1913						
March 28..	—	5	—	0.5 —	.....	.....

\* Five injections given.

CASE 557.—C. H. L., a man 50 years of age, with fairly well marked tabes dating back ten years since the onset. He had had marked gastric disturbances for the past three years, so that he had lost much weight and had been incapacitated for several months. In three and one-half months he received eleven intraspinal injections, a total of 130 c.c. undiluted serum, which was obtained from other patients. This treatment resulted in the reduction of all the abnormal constituents in the fluid nearly to normal. Then 1.5 gm. salvarsan in five intravenous injections was given with practically no effect on the fluid. The patient was so much improved that he returned to work and received no further treatment. At the time of the last examination of the fluid, nearly one year after treatment was discontinued, his spinal fluid was entirely normal. The tabetic process has shown practically no advance since the treatment was started.

TABLE 4.—No. 675. H. G. G. AGE 42. TABES DORSALIS TWO AND ONE-HALF YEARS. SYPHILIS DENIED. RESULT: IMPROVED

Date, 1912	Blood Wassermann Reaction	Cerebrospinal Fluid			Treatment
		Cells	Noguchi Globulin	Wass. Reaction c.c. Spinal Fluid	Intraspinal Other Patients' Serum
March 18....	±	35	±	0.3 ++	.....
May 2.....	—	..	..	.....	.....
May 5.....	..	41	+	0.3 ++	30 c.c. of 40%
May 14.....	..	24	±	0.4 ++	30 c.c. of 40%
May 21.....	..	17	±	0.4 ++	30 c.c. of 40%
June 10.....	—	..	..	.....	.....
June 20.....	..	14	±	0.5 ++	30 c.c. of 40%
June 27.....	..	33	±	0.5 ++	30 c.c. of 40%
July 9.....	..	12	±	0.5 ++	30 c.c. of 40%
July 21.....	..	35	+	0.5 ++	30 c.c. of 40%
July 30.....	—	..	..	.....	.....
Sept. 19.....	..	14	—	0.5 ±	30 c.c. of 40%
Oct. 1.....	..	8	—	0.5 —	30 c.c. of 40%
Oct. 19.....	..	7	—	0.5 —	30 c.c. of 40%
1913					
Jan. 5.....	—	5	—	0.5 —	.....

Died of lobar pneumonia. January 5. Autopsy showed typical tabes.

CASE 675.—H. G. G., a man 42 years of age, who had well marked tabes of two and one-half years' duration. He had never received any antisyphilitic treatment previous to his admission. In five and one-half months he was given ten intraspinal injections, a total of 120 c.c. of undiluted serum. After eight treatments the cerebrospinal fluid was normal. About three months after his last treatment he died of lobar pneumonia. The cerebrospinal fluid taken immediately after death was normal. Autopsy revealed changes in the cord typical of tabes dorsalis. The symptomatic improvement, as well as the return to normal of the cerebrospinal fluid, were most striking. This patient received no intravenous treatment at any time.

TABLE 5.—No. 565. V. H. AGE 42 YEARS. TERTIARY SYPHILITIC MENINGITIS (RADICULITIS). SYPHILIS NINE YEARS AGO

Date, 1912	Blood Wassermann Reaction	Cerebrospinal Fluid			Treatment Intraspinous Other Patients' Serum
		Cells	Noguchi Globulin	Wass. Reaction c.c. of Spinal Fluid	
April 5.....	+±	124	++	0.1 ++	.....
April 10.....	....	64	++	0.1 +±	30 c.c. of 40%
April 19.....	....	17	+	0.2 ++	30 c.c. of 40%
April 28.....	....	30	+	0.2 ++	30 c.c. of 40%
May 7.....	....	23	+	0.2 ++	30 c.c. of 40%
Sept. 12*....	....	4	—	—	.....

\* Report of findings on September 12 furnished by Neurological Institute.

CASE 565.—V. H., a man 42 years old, who had pains referable to dorsal root irritation for three years. One year before admission he had received two intravenous injections of salvarsan followed by a course of mercury inunctions and considerable potassium iodid by mouth. Before this treatment was started he had 94 cells per c.mm. in the cerebrospinal fluid. Our first examination showed 124 cells, a heavy globulin and a strongly positive Wassermann reaction. He then had 48 c.c. of serum in four intraspinous injections. Four months later he was examined in another hospital and his spinal fluid reported as normal. Unfortunately, the patient would not continue under observation. The symptomatic improvement was less marked than the improvement in the pathological cerebrospinal fluid.

TABLE 6.—No. 485. C. M. AGE 48. TABES DORSALIS ONE AND ONE-HALF YEARS. SYPHILIS NINETEEN YEARS

Date, 1912	Blood Wass. Reaction	Cerebrospinal Fluid			Treatment	
		Cells per c.mm.	Noguchi Globulin	Wassermann Reaction c.c. Spinal Fluid	Intra-venous Salvarsan. gm.	Intraspinous Serum
Jan. 26....	—	130	++	0.5 ++	.....	.....
Jan. 30....	—	90	++	0.5 +	0.2	30 c.c. of 50%
Feb. 6....	—	40	++	0.5 ±	0.3	.....
Feb. 13....	—	26	++	0.5 —	0.3	25 c.c. of 40%
Feb. 20....	—	24	++	0.5 —	0.3	30 c.c. of 40%
April 3....	—	9	+	0.5 —	0.3	20 c.c. of 40%
April 17...	—	...	....	.....	0.3	.....
May 6....	—	6	+	0.5 —	0.3	30 c.c. of 40%
June 20....	—	8	+	0.5 —	0.3	30 c.c. of 40%
July 18....	—	4	±	0.5 —	0.3	30 c.c. of 40%
Aug. 15....	—	4	±	0.5 ±?	0.3	30 c.c. of 40%
Sept. 12....	—	2	±	0.5 ±?	0.4	30 c.c. of 40%
Oct. 29....	—	...	....	.....	*0.9	.....
Nov. 19....	—	4	+	0.5 —	.....	.....
Dec. 17....	—	...	....	.....	*0.9	.....
1913						
April 9....	—	3	+	0.5 —	0.5	.....

\* Neosalvarsan.



These three cases illustrate very well that definite and marked change in the condition of the cerebrospinal fluid can be brought about by the intraspinal injection of serum alone, since none of these last three patients had had any other form of treatment. As there is also a definite effect from intravenous injection of salvarsan, it would seem that the combination of these two forms of treatment would produce a more rapid effect than either alone. This is well illustrated by the next three cases.

CASE 485.—C. M., a man aged 48 years, with moderately advanced tabes of two years' duration. He had transient diplopia seven years ago. In eight months patient was given 3.3 gm. salvarsan in eleven intravenous injections, and nine intraspinal injections, a total of 109 cubic centimeters of his own serum. After the fourth intraspinal treatment the fluid was practically normal except for a moderate increase in globulin which has persisted up to the present time.

TABLE 7.—No. 893. F. B. AGE 34. TABES DORSALIS TWO YEARS. SYPHILIS THIRTEEN YEARS. RESULT: IMPROVED

Date, 1912	Blood Wass. Reaction	Cerebrospinal Fluid			Treatment	
		Cells per c.mm.	Noguchi Globulin	Wassermann Reaction, c.c. Spinal Fluid	Intravenous Neosalvarsan, gm.	Intraspinal Serum
Oct. 18.....	+	40	±	0.5 +	0.75	.....
Oct. 28.....	++	...	..	.....	0.45	.....
Nov. 2.....	..	57	+	0.5 +±	.....	.....
Nov. 4.....	++	47	+	0.5 ++	0.9	30 c.c. of 40%
Nov. 12....	...	13	+	0.5 —	0.9	30 c.c. of 40%
Nov. 18....	...	...	..	.....	0.9	.....
Nov. 25....	++	...	..	.....	0.9	.....
Dec. 2.....	++	10	+	0.5 —	0.9	30 c.c. of 40%
Dec. 16....	...	...	..	.....	0.9	.....
Dec. 17....	...	2	±	0.5 —	.....	.....
1913	*			*	.....	.....
Jan. 25....	...	7	±	0.5 —	.....	.....
Feb. 8.....	++	5	±	0.5 —	0.9	30 c.c. of 40%
March 14..	++	...	..	.....	0.75	.....
March 28 to	++	...	..	.....	.....	.....
April 25...	++	...	..	.....	† 3	.....
April 25...	++	3	±	0.5 —	.....	.....

\* Cholesterin heart antigen.

† Salvarsan. Three injections given.

CASE 893.—F. B., a man 34 years of age, who had early tabes, suffering chiefly from shooting pain in the legs for two years, and slight unsteadiness in gait for six months. In four months he received 8.2 gm. neosalvarsan and four intraspinal injections, a total of 48 c.c. of his own serum. The increase in the intensity of the Wassermann reaction after the treatment was started is worthy of note. The sudden drop in cells and the disappearance of the Wassermann reaction occurred after the first intraspinal injection. After the fluid was reduced to normal except for a slight increase in globulin, the treatment was continued intravenously because of the persistence of the Wassermann reaction in the blood serum. This case illustrates the point which we have noticed, that

if, before treatment was started, large quantities of the fluid are required to obtain a positive Wassermann reaction, the reaction disappears fairly rapidly under treatment.

TABLE 8.—No. 605. F. K. AGE 27. CEREBROSPINAL SYPHILIS. DURATION (?)  
PREVIOUS TREATMENT NONE. RESULT: IMPROVED

Date, 1912	Blood Wass. Reaction	Cerebrospinal Fluid			Treatment	
		Cells per c.mm	Noguchi Globu- lin	Wassermann Reaction c.c. Spinal Fluid	Intra- venous Salvarsan, gm.	Intraspinal Serum
April 25...	++	59	+	0.2 ++	0.3	30 c.c. of 40%
May 2....	++	...	..	0.3 ++	0.3	30 c.c. of 40%
May 9....	+	...	..	.....	0.3	.....
May 23....	+	14	+	0.3 ++	0.3	30 c.c. of 40%
June 13....	+	5	±	0.3 ++	0.3	30 c.c. of 40%
June 20....	+	6	±	0.4 ++	0.3	30 c.c. of 40%
July 18....	±	2	±	0.5 ++	0.3	30 c.c. of 40%
July 26....	..	...	..	.....	0.3	.....
Aug. 15....	±	2	±	0.4 ++	0.3	30 c.c. of 40%
Sept. 12....	±	6	±	0.4 ++	0.4	30 c.c. of 40%
Oct. 3.....	±	...	..	.....	0.6	.....
Oct. 17....	—	...	..	.....	0.6	.....
Nov. 2....	..	7	—	0.5 ++	0.6	.....
Nov. 22....	+	...	..	.....	0.6	.....
Nov. 29 ..	..	...	..	.....	†0.9	.....
Dec. 12....	—	8	—	0.5 ++	†0.9	.....
1913	*			*		
Jan. 3....	++	4	—	0.5 ++	†0.9	30 c.c. of 40%
Jan. 31....	++	3	—	0.5 +±	†0.9	30 c.c. of 40%
Feb. 28....	+±	4	—	0.5 +	†0.9	30 c.c. of 40%
April 4....	+±	...	..	.....	0.6	.....
April 25...	+	2	—	0.5 —	0.5	30 c.c. of 40%

\* Cholesterin heart antigen.

† Neosalvarsan.

CASE 605.—F. K., a man 27 years of age, who was admitted because of ophthalmoplegia interna of the left eye. He denied syphilis and had not taken anti-syphilitic treatment. His physical examination was normal except for one dilated pupil. In one year he received twenty intravenous injections, a total of 6.6 gm. of salvarsan and 4.5 gm. neosalvarsan, and 144 c.c. of his own serum in twelve intraspinal injections. At the end of this time the cerebrospinal fluid became normal and there was a distinct diminution in the intensity of the Wassermann reaction in the blood-serum.

This is the type of case which Nonne and Erb have described as usually going on to tabes or paresis. The distinct changes which were first found in the cerebrospinal fluid indicate that the patient was suffering from an active syphilis of the central nervous system, although he presented as physical signs only one dilated pupil. It seems reasonable to suppose that the treatment with the resulting return to normal of the cerebrospinal fluid has resulted in an arrest of the syphilitic process.

Probably by careful clinical examination, as well as examination of the cerebrospinal fluids, many cases of tabes could be detected in this early stage and arrested by applying intensive and prolonged treatment. It is worthy of note that many of the tabetics give a history of diplopia or other transient eye disturbances several years before the onset of any other symptoms. It is important to recognize the disease in this early stage in order that it may not be allowed to progress until marked nerve degeneration has occurred.

TABLE 9.—No. 152. O. F. AGE 32. TABES DORSALIS THREE AND ONE-HALF YEARS. SYPHILIS TEN YEARS. RESULT: IMPROVEMENT, RELAPSE, IMPROVEMENT

Date, 1911	Blood Wass. Reaction	Cerebrospinal Fluid				Treatment	
		Cells per c.mm.	Noguchi Globulin	Wass. Reaction		Intra-venous Salvar-san, gm.	Intraspinous Serum
				Liver Antigen c.c. of Spinal Fluid	Cholesterin Heart Antigen c.c. of Spinal Fluid		
April 4 to.	++	27	++	0.1 —	.....	.....	.....
May 18....	+	...	...	.....	.....	5 x 0.2	.....
May 19....	....	39	++	0.1 +	.....	.....	.....
Oct. 13....	+	...	...	.....	.....	0.2	.....
Oct. 20....	±	9	+	0.1 ±	.....	0.2	17 c.c. of 40%
Oct. 27 to..	....	...	...	.....	.....	.....	.....
Nov. 17....	....	...	...	.....	.....	4 x 0.2	.....
Nov. 20....	....	10	+	.....	.....	.....	.....
1912							
April 9....	—	14	±	0.2 ++	.....	0.3	30 c.c. of 40%
April 15....	....	...	....	.....	.....	0.3	.....
April 23....	±	3	+	0.2 ++	.....	0.3	30 c.c. of 40%
April 30....	±	...	....	.....	.....	0.3	.....
May 7....	±	6	+	0.2 ++	.....	0.3	30 c.c. of 40%
Sept. 23....	±	19	+	0.3 ++	.....	§0.9	.....
Oct. 8....	±	...	....	... 0	.....	§0.9	.....
1913	*						
Jan. 31....	+±	70	+	0.2 ++	0.1 ++	§0.75	.....
Feb. 7....	+±	57	±	0.2 ++	0.2 ++	§0.9	30 c.c. of 40%
Feb. 14....	+±	28	±	0.3 ++	0.2 ++	§0.9	30 c.c. of 40%
Feb. 28....	++	17	±	0.3 ++	0.2 ++	§0.09	30 c.c. of 40%
March 14..	+	14	—	0.2 ++	0.2 ++	§0.75	30 c.c. of 40%
March 27..	++	7	+	0.5 ++	0.3 ++	§0.75	30 c.c. of 40%
April 9....	+±	7	+	0.5 ±	0.3 ++	§0.9	30 c.c. of 40%

\* Cholesterin heart antigen. § Neosalvarsan.

CASE 152.—O. F., a man 32 years of age, who illustrates both the result of intraspinous treatment and the necessity of continuing some form of treatment until the cerebrospinal fluid is normal. He had a well marked tabes of three and one-half years' duration, with severe shooting pains. Previous to his admission he had received very little anti-syphilitic medication. In the first year he received 2.2 gm. salvarsan in eleven intravenous injections, which

resulted in slight reduction in the degree of pleocytosis, a moderate reduction in globulin, and as far as could be determined, little if any effect on the Wassermann reaction in cerebrospinal fluid. Then in one month's time he had 1.5 gm. salvarsan in five intravenous injections plus three intraspinal injections, a total of 36 c.c. of his own serum. This resulted only in a reduction of the pleocytosis. For the next eight months he refused to have systematic treatment. In January, 1913, the pains became more severe and about the end of the month he developed diplopia. Examination of the cerebrospinal fluid revealed a more marked pleocytosis than on any previous examination. In the next two months systematic treatment consisting of seven intravenous injections, a total of 5.8 gm. neosalvarsan, and six intraspinal injections of his own serum, a total of 72 c.c., resulted in a reduction in cells to nearly normal, and a marked decrease

TABLE 10.—No. 113. W. L. S. AGE 52. TABES DORSALIS SEVEN YEARS. SYPHILIS TWENTY-THREE YEARS. IMPROVEMENT. RELAPSE

Date, 1911	Blood Wass. Reaction	Cerebrospinal Fluid			Treatment	
		Cells	Noguchi Globu- lin	Wass. Reaction, c.c. Spinal Fluid	Intra- venous Salvarsan, gm.	Intraspinal Serum
March 7...	++	72	++	0.1 —	.....	.....
March 11..	++	...	....	.....	0.35	.....
March 28..	++	27	++	0.1 —	0.5	.....
April 4....	+	...	....	.....	†	.....
May 22....	++	...	....	.....	†	.....
Oct. 5.....	++	...	....	.....	†	.....
Oct. 7 to...	±	...	....	.....	.....	.....
Nov. 4.....	±	...	....	.....	‡0.2	.....
Nov. 5.....	....	36	+	0.1 —	.....	.....
1912						
Jan. 5.....	+	24	++	0.4 ++	0.3	30 c.c. of 40%
Jan. 13....	+	30	+	0.4 ++	0.3	28 c.c. of 40%
Jan. 21....	++	10	++	0.4 ++	0.3	20 c.c. of 50%
Jan. 27....	+	8	++	0.5 ++	0.3	25 c.c. of 40%
Feb. 3.....	+	28	+	0.4 ++	0.3	30 c.c. of 50%
Feb. 10....	++	5	++	0.4 ++	0.3	30 c.c. of 50%
March 16..	+	9	+	0.5 +	0.3	30 c.c. of 40%
March 23..	+	3	+	0.4 ++	0.2	30 c.c. of 40%
April 6....	±	3	±	0.5 —	0.2	30 c.c. of 40%
April 13...	+	3	±	0.5 —	0.3	.....
May 27 to						
July 8.....				.....	§	.....
Sept. 13...	+	2	±	0.5 —	¶0.9	.....
Sept. 21....	±	...	....	.....	¶0.9	.....
Nov. 23....	....	6	+	0.5 —	.....	.....
1913	*			*		
Jan. 25....	++	9	±	0.5 —	¶0.6	.....
Feb. 8.....	±	...	....	.....	¶0.6	.....
Feb. 22....	++	...	....	.....	¶0.6	.....
March 8....	±	...	....	.....	¶0.75	.....
March 22...	±	...	....	.....	¶0.75	.....
April 5....	++	7	+	0.4 ±	¶0.6	.....

\* Cholesterin heart antigen. ‡ Five injections given.

† Mixed treatment by mouth.

§ Six injections Hg. salicylate intramuscularly.

¶ Neosalvarsan.



in the intensity of the Wassermann reaction in the cerebrospinal fluid. At the same time the pains and diplopia disappeared. The symptomatic relapse and the increase in the signs of the active pathological process as shown by the cerebrospinal fluid in the patient is in striking contrast to the subsequent course of the disease in those patients whose cerebrospinal fluid had been rendered normal by treatment.

CASE 113.—W. L. S., a man 52 years of age, who had a moderate tabes of seven years' duration. The principal symptom was shooting pain. In the first ten months of treatment he had seven intravenous injections of salvarsan, a total of 1.8 gm., and six months mixed treatment. This resulted in a reduction in cells from 72 to 36 per c.mm., and a moderate decrease in globulin. In the next three months he received 2.5 gm. salvarsan in nine intravenous injections and nine intraspinal injections of his own serum, a total of 109 c.c. At the end of this course of treatment the cerebrospinal fluid was normal except for a trace of globulin. Because of the persistence of a positive Wassermann reaction in the blood, intramuscular injections of mercury were given, but could not be tolerated long. Occasional lumbar puncture showed that the cells were increasing slightly in number, and systematic intravenous treatment with neosalvarsan was given. After five treatments, a total of 3.3 gm., it was found that the spinal fluid again showed a positive Wassermann reaction with 0.4 c.c. This returning reaction cannot be attributed to the increased sensitiveness of the cholesterin-heart antigen, because the reaction was negative in January with the same antigen. This patient has shown less symptomatic improvement than any of our other patients. After a course of treatment there would be some decrease in pain and periods of a week or more without pain, but no permanent relief has been obtained. The return of the positive Wassermann reaction in the cerebrospinal fluid seems to indicate that the syphilitic process is still active in some part of the cerebrospinal axis. The disappearance of the reaction under combined intraspinal and intravenous treatment, and its reappearance under intravenous treatment alone, seems to point to the advantage of the combined method in this patient at least.

#### DISCUSSION

The cases cited above are only examples of the various groups of patients whom we have under treatment. From the experience of the past two years we now feel that 0.2 gm. or 0.3 gm. salvarsan is too small a dose, and that neosalvarsan given in the same relative amounts as salvarsan is much inferior to salvarsan. At present we are giving full doses of salvarsan—0.45 gm. to 0.5 gm.—every two weeks, and in addition intraspinal injections of 30 c.c. of 40 per cent. serum, until the cerebrospinal fluid shows a normal cell count and a negative Wassermann reaction. Under this plan of treatment many of the cases are showing more rapid improvement than did those treated a year or more ago, illustrated in these tables. Unfortunately, our experience with general paralysis is too limited to give definite results. One patient with early paresis and one or two others who are border-line cases between tabes and paresis have shown a rapid decrease in pleocytosis and a moderate decrease in the globulin, but the Wassermann reaction has been slower in showing a response. When one considers the anatomical difficulties in reaching the myriads of spirochetes that are present in the brain in this condition, he is impressed with the necessity of prolonged and vigorous treatment.

Another point brought out by the work here presented, is that the treatment of chronic syphilitic diseases of the central nervous system is not the administration of two or four intravenous injections of salvarsan, or the exhibition of a certain amount of mercury and iodids. Each case presents a slightly different problem, and must be considered individually. Some respond much more rapidly than others, but the object in all should be to obtain a persistently normal cerebrospinal fluid. Of course, we cannot state with certainty that a normal cerebrospinal fluid assures that there will be no further progress of the degenerative process. All we can say is that as long as the cerebrospinal fluid gives evidence of a specific pathological process in the central nervous system, and we have specific therapeutic measures which will remove that evidence, these measures should be applied. Only when the fluid has become normal are we justified in discontinuing the treatment, and observations of the fluid should be continued so that at the first evidence of relapse treatment may be resumed.

The method of intraspinal injection is not presented as a substitute for any of the accepted forms of treatment, but as an aid in attacking these severe infections. We feel that there is definite evidence that this form of treatment has a curative action on the syphilitic process, and that, therefore, its combination with intensive intravenous treatment is indicated where specially intensive treatment is required, as in rapidly advancing tabes or paresis, or where the disease has resisted other forms of treatment.

## THE VALUE OF EDESTIN AND PEPTONE IN THE DIAGNOSIS OF CANCER OF THE STOMACH \*

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Neubauer and Fischer,<sup>1</sup> in 1909, proposed the use of glycytryptophan as a reagent for the estimation of peptolytic activity and applied it to the diagnosis of carcinoma of the stomach. The test consisted in mixing glycytryptophan with a portion of the filtered gastric juice obtained after the usual test breakfast, incubating for twenty-four hours, and then testing with bromin vapor for the rose-violet color of free tryptophan.

Their conclusions were as follows:

1. There exists in carcinomatous stomach contents a ferment which, contrary to pepsin, will split glycytryptophan.
2. This ferment is destroyed by an acidity of .36 per cent. HCl.
3. The presence of this ferment may be of diagnostic value.

Their report was accepted with widely divergent criticism. Lyle and Kober,<sup>2</sup> Pechstein,<sup>3</sup> Oppenheimer<sup>4</sup> and others, considered it of value. Ley,<sup>5</sup> Ehrenberg,<sup>6</sup> Sanford and Rosenbloom,<sup>7</sup> etc., believed it valueless. Several authors described modifications of the original method. Weinstein<sup>8</sup> tested stomach contents directly for free tryptophan; Kuttner and Pulvermacher<sup>9</sup> used silk peptone as a less expensive reagent, estimating peptolytic action by the precipitation of tyrosin under the microscope; Jacque and Woodyatt<sup>10</sup> and Schryver and Singer<sup>11</sup> proposed a quantitative test, applying the formol titration of Sorensen-Ronchese to a mixture of gastric juice and Witte peptone. None of these modifications, with the exception of the last-named, have yielded more uniform results than the original method. In the procedure about to be described we have used the peptone quantitative method and have added the use of edestin

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\* From the Department of Clinical Research, Michael Reese Hospital, and the Morris Institute for Medical Research.

1. Neubauer and Fischer: *Deutsch. Arch. f. klin. Med.*, 1909, xcvi, 499.
2. Lyle and Kober: *New York Med. Jour.*, 1910, xci, 1151.
3. Pechstein: *Berl. klin. Wehnschr.*, 1911, xlviii, 375.
4. Oppenheimer: *Deutsch. Arch. f. klin. Med.*, 1910-11, ci, 293.
5. Ley: *Berl. klin. Wehnschr.*, 1911, xlviii, 119.
6. Ehrenberg: *Berl. klin. Wehnschr.*, 1911, xlviii, 704.
7. Sanford and Rosenbloom: *THE ARCHIVES OF INT. MED.*, 1912, ix, 445.
8. Weinstein: *Jour. Am. Med. Assn.*, 1911, lvii, 1420.
9. Kuttner and Pulvermacher: *Berl. klin. Wehnschr.*, 1910, xlvii, 2057.
10. Jacque and Woodyatt: *THE ARCHIVES INT. MED.*, 1912, x, 560.
11. Schryver and Singer: *Quart. Jour. Med.*, 1912, vi, 71.

as a means of controlling the most frequent source of error — proteolytic cleavage.

One of the most frequent criticisms of the biochemical tests for cancer of the stomach is the fact that the gastric contents of normal individuals, as well as of non-cancerous patients, under certain conditions, split glycyltryptophan and other polypeptids. Although several authors have defined the sources of error in these tests and have suggested various modifications to control them, no one, so far as we are aware, has made use of a second substrat to control and rule out the cleavage due to non-cancerous ferments. In view of these considerations, we propose, therefore, to discuss, first, the probable sources of error; second, to submit a method for the control of the most frequent error — proteolytic cleavage — third, to report a series of cases in which this method has been used.

1. *The sources of error in the ability of gastric contents to split polypeptids in the absence of malignant disease.*

Emerson,<sup>12</sup> following the suggestion of Friederich Müller, showed that the unusually deep cleavage of proteins by gastric contents of cancer patients was due to the activity of a ferment derived from the cancer cells. This ferment Abderhalden<sup>13</sup> believed belonged to the general group of ereptases, ferments capable of splitting polypeptids and peptones, but incapable of attacking native protein. While minor differences have been found to exist between cancer ereptase and the universal ereptases of normal tissues, no specificity has been noted so far as the cleavage of polypeptids and peptone is concerned. It has been shown further by one of us,<sup>14</sup> that quantitatively cancer cells of the epithelioid type contain less ereptase than normal kidney and liver cells (dog), but more than normal spleen and blood serum; while cancers of the connective tissue type contain least of all. It should be emphasized, therefore, that these tests based on peptolytic cleavage are specific only insofar as the ereptase of any given gastric contents can be shown to be derived from the cancer tissue itself. To do so, we must rule out ereptase from non-cancerous sources, as follows:

(a) The ereptase (erepsin) of regurgitated duodenal contents (succus entericus).

(b) Serum ereptase from hemorrhage into the stomach and from transudation of ereptase-containing fluids into the stomach.

(c) Tissue ereptase from breaking down of cells of the gastric mucosa.

(d) Ereptase of swallowed saliva.

In addition, protease, capable of peptolytic cleavage, as well as proteolytic cleavage, must be identified and controlled, if present:

(a) Trypsin of regurgitated pancreatic juice.

(b) Proteases of bacteria and leukocytes — mostly from swallowed saliva.

12. Emerson: *Deutsch. Arch. f. klin. Med.*, 1902, lxxii, 415.

13. Abderhalden: *Ztschr. f. Krebsforsch.*, 1910, ix, 226.

14. Hamburger: *Jour. Am. Med. Assn.*, 1912, lix, 847.



From a general survey of these several sources of error, we considered, for reasons to be discussed later, that the second group, the proteases of pancreatic juice (trypsin) and saliva (leukocytes and bacteria), were most frequently active. We believed, therefore, that if this proteolytic cleavage could be controlled, we should materially increase the reliability of the methods in question. Our results (described in Section 3) would lend support to this view.

The presence of pancreatic trypsin as a disturbing factor in the gycyltryptophan test was recognized by Neubauer and Fischer in their original publication and was controlled by discarding bile-stained gastric contents.

Kuttner and Pulvermacher, Lyle and Kober, Öhrl and Schittenhelm<sup>15</sup> and others, contended subsequently that the bile test was inadequate to exclude trypsin. Boldyreff has shown recently that a yellow, clear fluid — *free from bile*, rich in carbonates (pancreatic juice) and containing pancreatic ferments, flowed from the gastrostomy tube of a Pawlow dog, following the withdrawal of a weak hydrochloric acid solution, introduced for the purpose of stimulating the secretion of pancreatic juice. On the basis of our own work we agree with those holding that a negative bile test does not of itself exclude trypsin.

Warfield<sup>16</sup> and Koehlker<sup>17</sup> first called attention to the fact that saliva under certain conditions is capable of hydrolyzing gycyltryptophan and other di- and tripeptids, believing such action to be due to a hitherto undescribed salivary ferment. Jacque and Woodyatt and Smithies,<sup>18</sup> experimenting with saliva after passage through a Berkefeld filter and centrifuging, conclude that the peptolytic action is due, at least in large part, to bacteria. From certain experiments of our own, we have found in various salivas evidences of proteolytic (as well as peptolytic) activity, due probably to the contained leukocytic ferments (leukoprotease). This leukoprotease we believe to be responsible for much of the salivary peptolytic action. We would further emphasize that the method about to be described for the control of pancreatic trypsin serves equally well for salivary leukoprotease, thereby dismissing, for purposes of clinical diagnosis, the problem of the origin of the proteolysis.

*2. The use of edestin and peptone in differentiating and estimating proteolytic and peptolytic cleavage.*

The use of the vegetable globulin edestin was suggested to us by Dr. Jobling as a true native protein subject to cleavage by proteolytic fer-

15. Öhrl and Schittenhelm: Zentralbl. f. d. gesamt. Physiol. u. Path. d. Stoffwechs., 1912, xi, 881.

16. Warfield: Bull. Johns Hopkins Hosp., 1911, p. 150.

17. Koehlker: Ztschr. f. physiol. Chem., 1911, No. 30.

18. Smithies: THE ARCHIVES INT. MED., 1912, x, 521.

ments only, thereby serving as a substrat for differentiating cleavage due to peptolytic enzymes. Casein, which was used to some extent in our earlier experiments, was later discarded in view of its slight digestion by erepsin (Cohnheim). Fischer and Abderhalden<sup>19</sup> and Abderhalden and Gigon<sup>20</sup> have previously used edestin in estimating the activity of pancreatic juice and ferments, while Fuld and Levison<sup>21</sup> have used it for quantitative determinations of pepsin. These several procedures are based on the fact that edestin is soluble in both acids and alkalies, but is insoluble in neutral solutions, particularly in the presence of concentrated salt solution. (Osborne.)

#### METHOD

(a) *Control and Estimation of Proteolytic Cleavage (Proteolysis).*—Crystallized edestin, prepared by macerating hemp seeds in 10 per cent. NaCl, then dialyzed and precipitated with 95 per cent. alcohol, is made up as a 0.1 per cent. solution in 0.1 per cent.  $\text{Na}_2\text{CO}_3$ . This stock solution in the refrigerator remains unchanged for several days to a week.

The filtered (through ordinary moistened filter paper) gastric contents, obtained as soon as possible after the usual Ewald breakfast, are neutralized with a normal  $\text{Na}_2\text{CO}_3$  solution (2 drops of a 1 per cent. alcoholic phenolphthalein solution serving as indicator) and brought to an alkalinity equal to N/100  $\text{Na}_2\text{CO}_3$ , for the purpose of inactivating pepsin while activating trypsin and other alkaline and neutral acting proteases.

Two c.c. of the edestin solution is placed in each of four ordinary test-tubes, and the neutralized gastric filtrate added in decreasing amounts of 2 c.c., 1 c.c., 0.5 c.c., respectively, to the first three test-tubes; the fourth tube, containing edestin and carbonate alone, serving as control. All four tubes are made up to an equivalent volume with a 1 per cent.  $\text{Na}_2\text{CO}_3$  solution. The tubes are then placed in the incubator at 37 C., and removed at the end of four hours. Proteolysis is estimated by the precipitation of unhydrolyzed edestin by the drop by drop addition of 5 per cent. acetic acid; complete absence of turbidity on neutralization indicating complete digestion; slight turbidity, partial digestion; maximum turbidity (as compared with the control tube), no digestion. The degree of proteolysis is indicated in the protocols as follows: Complete digestion in all tubes (except control) (no precipitate on neutralization), + + +; partial digestion (no precipitate in Tubes 1 and 2, with turbidity in Tube 3), + +; minimal digestion (no precipitate in Tube 1, with turbidity in 2 and 3), +; no digestion (turbidity in all tubes), 0.

19. Fischer and Abderhalden: *Ztschr. physiol. Chem.*, 1905, xlv, 52.

20. Abderhalden and Gigon: *Ztschr. physiol. Chem.*, 1907, liii, 119.

21. Fuld and Levinson: *Biochem. Ztschr.*, 1907, vi, 473.

Care is necessary that only the minimum of acid necessary to neutralize be added, as excess of acid causes a re-resolution of edestin. It is well to standardize the number of drops of acid necessary for neutralization in the control tube with the aid of a drop of phenolphthalein.

(b) *Estimation of Peptolytic Cleavage (Peptolysis).*—Ten c.c. of the gastric filtrate are added to 20 c.c. of a 2 per cent. Witte's peptone solution and 10 c.c. of the mixture removed as control and titrated for free amino groups by the Sorensen-Ronchese formol method, as applied by Jacque and Woodyatt. The remaining 20 c.c. are placed in an Erlenmeyer flask under toluol at 37 C. for twenty-four hours; at the end of that period, 10 c.c. are again removed and titrated, the difference between the first and the second titration expressing the degrees of peptolysis in terms of c.c. N/10 NaOH per 100 c.c. of the mixture.

### 3. Results of the application of the edestin peptone method in a series of cases.

This report comprises the results obtained from thirty-seven cases, divided as follows:

	Cases
(a) Cancer of the stomach.....	10
(b) Chronic ulcer of the stomach.....	5
(c) Chronic inflammation of gall-bladder and pancreas.....	6
(d) Old gastro-enterostomy cases .....	4
(e) Control cases .....	12
Total .....	37

Only those cases are included in which the diagnosis was controlled by the operative or autopsy findings, or in which the clinical picture was so definite as practically to exclude errors in its interpretation.

Our conclusions may be summarized as follows, and we would emphasize that they are submitted as preliminary only, as they are drawn from too small a number of cases to be considered as final.

(a) *Cancer of the Stomach* (Table 1).—Moderately advanced and advanced cancer of the stomach, involving the pyloric portion, associated with low total acidity and low or absent free HCl produces greatest peptone cleavage (peptolysis 8.2; proteolysis trace).

Early cancer, situated on the lesser curvature between pylorus and cardia, possibly developing on an ulcer basis, with high total acidity and low or absent free HCl, gives least peptone cleavage (peptolysis 2.0; proteolysis 0).

Microscopic and chemical tests for bile are not sufficient to rule out tryptic proteolysis in all cases. Edestin is a reliable means of controlling proteolytic cleavage from pancreatic juice and saliva.

TABLE 1.—CANCER CASES. (TEN CASES.)

Case No.	Ewald						Peptone			Edestin		Diagnosis
	Amt. c.c.	Free HCl	Total Acid	Lactic	Bile	Blood	Control	Twenty-four Hour	Inc.			
1	110	0	9	0	0	0	10	18	8	*		Refused operation. Clinical diagnosis carcinoma; hard, palpable tumor mass.
2	110	0	8	0	0	0	10	25	15	*		Operation. Carcinoma, involving pylorus and one-third greater curvature. Pylorus partially stenosed.
3	70	0	20	++	0	0	13	19	6	*		Operation. Carcinoma of pylorus and greater and lesser curvature. Pylorus completely stenosed by foreign body.
4	130	0	42	0	0	0	10	12	2	*		Operation. Interosteal carcinoma with perforation; lesser curvature. Probable cancer on old ulcer.
5	150	0	12	++	0	†	11	22	11	++		Refused operation. Clinical diagnosis, carcinoma.
6	200	0	6	+	++	0	11	26	15	++		Operation. Carcinoma anterior wall near lesser curvature.
7	275	12	40	0	0	++	..	..	..	....		Operation. Carcinoma greater part lesser curvature and posterior wall; probable development of carcinoma from preceding gastric ulcer.
8	200	8	37	0	0	+	13	13	0	0		Operation. Carcinoma anterior wall of stomach developing from gastric ulcer. Mistaken diag. No evidence of malignancy (?)
9	120	0	6	+	0	0	7	15	8	0		Operation refused. Clinical diagnosis, carcinoma. Operation for stenosing carcinoma of pylorus and posterior wall and lesser curvature.
10	?	0	32	+	0	0	15	30	15	*		

\* Not made. † Trace. ‡ Vomitus.

TABLE 2.—ULCER CASES. (FIVE CASES)

Case No.	Ewald						Peptone			Edestin		Diagnosis
	Amt. c.c.	Free HCl	Total Acid	Lactic	Bile	Blood	Control	Twenty-four Hour	Inc.			
11	140	62	79	0	0	0	9	9	0	0		Duodenal ulcer.
12	130	0	8	+	†	0	18	18	0	++		Operation; chronic ulcer at duodenal pylorus juncture. No evidence of malignancy.
13	210	26	42	0	0	0	11	12	1	0		Chronic pyloric ulcer.
14	100	35	68	0	0	0	15	16	1	*		Chronic duodenal ulcer.
15	80	44	100	0	0	0	15	16.5	1.5	*		Chronic gastric ulcer.

\* Not made.



Lactic acid is not a marked inhibiting agent of peptolysis; blood, high total acidity and moderate free HCl (6 to 20) cause greatest (to complete) inhibition.

(b) *Chronic Ulcer of the Stomach and Duodenum* (Table 2).—Chronic ulcer associated with high free HCl and high total acidity produces practically no proteolytic or peptolytic cleavage (peptolysis 0.5; proteolysis 0).

In one instance (Case 12) chronic ulcer with absent free HCl and a total acidity of 8 gave no peptolytic cleavage, but positive proteolytic digestion.<sup>22</sup>

TABLE 3.—GALL-BLADDER AND PANCREAS CASES. (SIX CASES)

Case No.	Ewald						Peptone			Edestin	Diagnosis
	Amt. c.c.	Free HCl	Total Acid	Lactic	Bile	Blood	Control	Twenty-four Hour	Inc.		
16	25	0	25	0	+	0	11	14	3	*	Refused operation. Chronic cholecystitis and cholelithiasis.
17	90	0	11	0	0	0	11	24	13	++	Refused operation. Alcoholic gastritis; chronic cholecystitis.
18	?	0	10	+	0	0	9	20	11	+++	Operation. Chronic cholecystitis and pancreatitis.
19	50	0	34	0	0	0	9	15	6	++	Refused operation. Cholecystitis and cholelithiasis.
20	60	0	19	0	0	0	14	30	16	*	Refused operation. Chronic cholecystitis and gastritis.
21	50	0	10	+	0	0	11	24	13	+	Operation. Chronic cholecystitis with cholelithiasis; pancreatitis.

\* Not made.

(c) *Chronic Inflammations of Gall-Bladder and Pancreas* (Table 3).—Chronic cholecystitis and pancreatitis associated with absent free HCl and low or moderate total acidity, with negative tests for bile, cause both marked proteolysis and peptolysis (peptolysis 11 to 14.5; proteolysis + to +++).

In such cases edestin is an effective control of proteolytic cleavage, in particular where the clinical and laboratory findings point strongly to cancer. In our thirty-seven cases, six showing marked peptolysis with negative bile tests were so controlled by edestin, thereby reducing a possible 16 per cent. error to zero.

22. A larger series may show this to possess differentiating value between ulcer and early cancer, viz: absent peptolysis with positive proteolysis (with low acidities) speaking for ulcer; moderate peptolysis with moderate or absent proteolysis speaking for cancer.

(d) *Old Gastro-Enterostomy Cases* (Table 4).—In old gastro-enterostomy cases (for benign pyloric obstruction) with positive bile tests, proteolysis and peptolysis are both marked (peptolysis, 16.6; proteolysis, + + +).

It is of interest to note the parallel tests of bile and edestin as controls of proteolytic cleavage in this series, as compared with the negative bile and positive edestin tests of Table 3. It would appear that either bile experiences less difficulty in gaining entrance to the stomach through the gastro-enterostomy opening, or, and more likely, the stimulus to biliary secretion is increased by certain physiologic changes incident to the deflection of the chyme stream.

(e) *Control Cases* (Table 5).—Series of control (normal) cases show uniform absence of proteolysis and peptolysis in presence of free HCl, and in some cases positive proteolysis with negative bile tests (peptolysis, 0 to 4; proteolysis, 0 to +).

The degree of peptolysis and proteolysis in these several groups of cases may be tabulated as follows:

	Peptolysis Average	Proteolysis Average
<i>Cancer</i> (10 Cases)—		
7 Pyloric carcinomas .....	8.2	trace
2 Interosteal carcinomas .....	2.0	0
1 Lesser curv. and post. wall.....	0.0	0
<i>Ulcer</i> (5 Cases)—		
3 Chronic pyloric ulcers.....	0.5	+
2 Chronic duodenal ulcers.....	0.5	0
<i>Gall-Bladder and Pancreas</i> (6 Cases)—		
2 Chronic cholecystitis with cholelithiasis .....	4.5	+
2 Chronic cholecystitis with gastritis.....	14.5	++
1 Chronic cholecystitis with pancreatitis .....	11.0	+++
1 Chronic cholelithiasis with pancreatitis .....	13.0	+
<i>Gastro-Enterostomy</i> (4 Cases)—		
3 Old incompetent gastro-enterostomies for gastric ulcer with pyloric stenosis .....	16.6	+++
1 Old gastro-enterostomy or duodenal ulcer .....	0.	++
<i>Control Cases</i> (12 Cases)		
5 Neurasthenia, sec. anemia, gastropotosis. etc. ....	0.	0
7 Pernicious anemia, Basedow, acute nephritis, etc. ....	4.	+

These results may be taken to indicate that in cases of doubtful diagnosis in which cancer is being considered, a relatively high peptolysis with low proteolysis speaks for carcinoma; high peptolysis and high proteolysis against carcinoma.

TABLE 4.—GASTRO-ENTEROSTOMY CASES. (FOUR CASES)

Case No.	Ewald						Peptone			Edestin	Diagnosis
	Amt. c.c.	Free HCl	Total Acid	Lactic	Bile	Blood	Control	Twenty-four Hour	Inc.		
22	180	29	56	0	+++	0	12	12	0	++	Old, incompetent gastro-enterostomy for chronic duodenal ulcer. Old, incompetent ("vicious circle") gastro-enterostomy for gastric ulcer. Old, post gastro-enterostomy for pyloric ulcer. Sarcoma of intestine. Old (eighteen months), gastro-jejunostomy for supposed pyloric ulcer. Operation disclosed a carcinomatous ulcer in upper jejunum, opposite gastro-jejunal opening.
23	120	0	4	0	++	0	13	34	21	+++	
24	75	0	16	0	++	0	11	35	24	++	
25	80	37	62	0	++	0	7	12	5	++	

\* Not made.

TABLE 5.—CONTROL CASES. (TWELVE CASES)

Case No.	Ewald						Peptone			Edestin	Diagnosis
	Amt. c.c.	Free HCl	Total Acid	Lactic	Bile	Blood	Control	Twenty-four Hour	Inc.		
26	...	..	..	..	....	..	6	10	4	*	Pernicious anemia. Severe secondary anemia from unknown cause. [Pernicious anemia (?).]
27	50	12	38	X	0	0	9	18	9	+	
28	100	20	45	0	0	0	16	16	0	0	Gastroptosis; secondary anemia; chronic interstitial nephritis. Membranous colitis.
29	130	9	25	0	0	0	10	10	0	0	
30	...	13	43	..	....	..	..	..	..	....	Thrombo-angiitis obliterans. (Splanchnic vessels.)
30	40	18	36	0	0	0	11	11	0	*	
31	15	0	15	0	0	0	13	16	3	+	Membranous colitis. Neurasthenia.
32	110	25	66	..	....	..	15.5	16	1.5	*	
33	...	?	..	..	....	..	12	15	3	*	Refused operation. Carcinoma descending colon; enteroptosis. Exophthalmic goiter.
34	97	0	13	0	0	0	12	19	7	++	
35	...	..	?	..	....	..	11	13	2	+	Acute nephritis. Gastric neurosis.
36	160	..	?	..	....	..	11	14	3	*	
37	...	..	?	..	....	..	13	14	1	*	

\* Not made.

## SUMMARY

1. Edestin is a valuable aid in controlling the proteolytic cleavage of stomach contents.

2. The proteolytic cleavage of stomach contents is due in most instances to regurgitated trypsin, although leukocytes and bacteria probably play some rôle.

3. By the use of edestin with peptone it is possible to materially reduce the errors in non-cancerous and normal cases due to trypsin, leukocytes and bacteria.

4. The edestin-peptone method possesses distinct value in the diagnosis of cancer of the stomach and is of considerable service in the differential diagnosis between benign and malignant anacidity.

5. High peptolysis with low proteolysis speaks for carcinoma; high peptolysis with high proteolysis against carcinoma.

6. The edestin-peptone method, as in other laboratory tests, is of practical value only when taken in conjunction with the usual clinical and laboratory findings.

The cases in this report were taken for the most part from the regular medical and surgical services of Michael Reese Hospital. Our thanks are given to the attending men and interns on the services for their cooperation and courtesy in placing these cases at our disposal. We are likewise indebted to Dr. Julia Crotty, of the Cook County Hospital, for two cases from the medical wards of that hospital. Finally, we would express our appreciation to Dr. Jobling for helpful criticisms and suggestions.



## BOOK REVIEW

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TUBERCULIN IN DIAGNOSIS AND TREATMENT. By Louis Hamman and Samuel Wolman. D. Appleton & Co., New York and London, 1912.

During the last ten years the use of tuberculin in diagnosis and treatment has received wide attention, but so numerous are the preparations of tuberculin now available, so varied their methods of application, and so diversified the views as to the choice of preparation, the value of tuberculin in diagnosis and its place as a curative measure, that it requires much time and accurate knowledge of the voluminous literature to acquaint oneself with the necessary details and current opinions. All this accumulated and valuable knowledge Hamman and Wolman have sifted and arranged, together with their own extensive experience, into a very interesting and exceedingly useful book. The subject is treated from its broadest and most rational aspect, and presented in a thorough and impartial manner.

The book of some three hundred and fifty pages is divided into three parts: the first deals with "The scientific principles underlying the diagnostic and therapeutic use of tuberculin"; the second with "The use of tuberculin in diagnosis," and the third with "The use of tuberculin in treatment." The first of these parts or chapters embodies an excellent résumé of the experimental basis for the practical application of tuberculin, and the theoretical problems connected therewith, and includes an interesting account of the gradual development of our knowledge concerning the tuberculin reaction and its relation to anaphylaxis. In the second part the various methods of applying tuberculin for diagnostic purposes are carefully described and the practical worth of these different methods analyzed and compared. Hamman and Wolman speak quite favorably of the conjunctival reaction, which in their hands has not been attended with any alarming results and which they think if used properly is harmless. They consider it the least sensitive test for the determination of the presence of a tuberculous focus in the body, but the most valuable positive test for determining the presence of an active tuberculous infection. The cutaneous and intracutaneous tests are so delicate that they can be used only to exclude the presence of tuberculosis. In the last part there is a thorough discussion of tuberculin as a therapeutic measure. The authors believe that it has value analogous to fresh air, rest and diet, and since it is an adjuvant if used properly, its worth should not be neglected. There follow descriptions of the different tuberculins now in use, their method of application in tuberculosis of different organs and the dangers dependent on their misuse.

The book represents a most careful and critical survey of the whole subject which is presented in an interesting and really illuminating manner.

# The Archives of Internal Medicine

Vol. XII

OCTOBER, 1913

No. 4

## ATYPICAL TYPHOID INFECTION \*

W. T. CUMMINS, M.D. AND P. K. BROWN, M.D.

SAN FRANCISCO

Typhoid fever with its well-known clinical and pathological picture may present itself in various atypical forms. Louis, in 1829, was apparently the first observer to note the disease without the usual intestinal lesions. Dittrich (1846), Lebert (1858) and Guyot (1870) contributed additional cases to the literature. Since the typhoid bacillus was not discovered and its etiological significance established by Eberth and Gaffky until 1880 and 1884, these early cases are open to question. Morre,<sup>1</sup> in 1880, reported a case of splenotyphoid with suppurative changes in the spleen and no demonstrable intestinal lesions. One year later Mader<sup>2</sup> reported a case of bronchotyphoid, while Church,<sup>3</sup> in 1882, reported three cases of extra-intestinal type. Among many others specifically described below, Vaillard<sup>4</sup> (1890), Phillips<sup>5</sup> and Beatty<sup>6</sup> (1897), Lartigau,<sup>7</sup> Richardson,<sup>8</sup> Howard<sup>9</sup> and Bryant<sup>10</sup> (1899), Turney<sup>11</sup> (1900),

\* Submitted for publication June 8, 1913.

\* Read at the meeting of the California State Medical Society, Oakland, April 17, 1913.

1. Morre: Enteric Fever without Disease of the Glands of the Ilium, Proc. Path. Soc., Dublin, 1880-82, ix, 26.

2. Mader: Tod. Bericht der k. k. Krankenanstalt Rudolf-Stiftung, Wien, 1881, p. 278.

3. Church: St. Barth. Hosp. Rep., 1882, xvii, 97.

4. Vaillard: De l'infection par le bacille typhique sans lésions intestinales, La Semaine méd., 1890, No. 12, p. 96.

5. Phillips: Proc. Clin. Soc., London, Feb. 12, 1897.

6. Beatty: Enteric Fever without Intestinal Lesions, Brit. Med. Jour., Jan. 16, 1897, p. 148.

7. Lartigau: On Typhoid Septicemia with a Report of two Cases, One of Which Was a Typhoid Infection without Intestinal Lesions, New York Med. Jour., xx, No. 158; Bull. Johns Hopkins Hosp., 1899, x, 55.

8. Richardson: Boston Med. and Surg. Jour., 1899, p. 498.

9. Howard: Extra-Intestinal Inflammatory Lesions Caused by Typhoid Bacillus, Philadelphia Med. Jour., 1899, p. 402.

10. Bryant: Brit. Med. Jour., 1899, i, 776.

11. Turney: A Case of Typhoid Septicemia; necropsy. Lancet, London, Sept. 29, 1900, p. 940.

Opie and Bassett<sup>12</sup> (1901), McDaniel<sup>13</sup> (1902), Kaufmann<sup>14</sup> (1911), and Posselt<sup>15</sup> (1912) have reported cases, all being confirmed by bacteriological or serological examination.

*Spleen.*—Doglietti<sup>16</sup> has noted a case of so-called splenotyphoid with the spleen three times larger than normal and without intestinal or mesenteric node involvement. Blood-cultures were negative, but the typhoid bacillus was isolated from the roseola. Blumenthal<sup>17</sup> observed marked lymphoid hyperplasia of the spleen together with infarction and no specific intestinal lesions. In this group may be placed the cases of Griffon<sup>18</sup> (streptococcus mixed-infection), Monroe and Campbell<sup>19</sup> (perisplenic abscess and empyema), Melchior<sup>20</sup> (splenic abscess), Karlinski<sup>21</sup> (myomalacia cordis-infarct, etc.), Cheadle<sup>22</sup> and Guizzetti.<sup>23</sup>

*Liver.*—Some observers have stated that typhoid fever may induce severe parenchymatous changes together with cirrhosis, necrosis and atrophy, the condition simulating acute yellow atrophy. In 1865, Politzer noted in a four-months old baby acute liver atrophy with distinct hyperemia and enlargement of the solitary follicles and Peyer's patches, as well as the mesenteric nodes. Powell<sup>24</sup> has reported the case of a child with typhoid fever, hepatic cirrhosis and death from acute yellow atrophy. There was, however, no report of bacteriological or serological findings. Tecklenburg<sup>25</sup> observed a case of acute yellow atrophy presenting all the features of typhoid fever. Marked parenchymatous hepatic changes were noted by Bruno<sup>26</sup> in a 50-year-old woman with headache, delirium, Kernig's sign, splenic enlargement, muscular contractures and Widal positive 1:60. Necropsy showed engorgement of cranial sinuses, cloudy meningeal fluid and no intestinal lesions. Of McCrae's<sup>27</sup> series of 105 necropsies, one showed liver abscess. A number of observers have reported this lesion, among them being Meyer,<sup>28</sup> who, in 1911, reported

12. Opie and Bassett: Bull. Johns Hopkins Hosp., July, 1901.

13. McDaniel: Isolation of *B. typhosus* from Unusual Localizations. Cholecystitis, Meningitis and a Five Month Fetus, Jour. Am. Med. Assn., 1902, xxxvii.

14. Kaufmann: Lehrb. der spez. path. Anat., i, 504.

15. Posselt: Atypische typhusinfektionen. Typhus ohne Darmerkrankungen, Lubarsch and Ostertag's Ergebn. d. allg. Path., 1912, i, 237.

16. Doglietti: Gazz. med. di Torino, 1895.

17. Blumenthal: Ein Beitrag zur path. Anat. des Paratyphus, Centralbl. f. Bakteriologie. (Orig.), 1903, xxxiv, 113.

18. Griffon: Bull. Soc. anat. de Paris, 1902, iv, 825.

19. Monroe and Campbell: Glasgow Med. Jour., 1904, lxii, 120; Glasgow Path. and Clin. Soc., May 9, 1909.

20. Melchior: Berl. Klin., 1909, No. 255.

21. Karlinski: Wien. med. Wchnschr., 1891, No. 11.

22. Cheadle: Lancet, London, 1897, ii, 254.

23. Guizzetti: Clin. med. ital., 1900, No. 6, p. 358.

24. Powell: Brit. Med. Jour., ii, 1086.

25. Tecklenburg: Inaug.-Dissert., Freiburg, 1902.

26. Bruno: Il Policlin., 1903, xxxiii, 1025.

27. McCrae: Osler's Modern Medicine, ii, 100.

28. Meyer: Wissensch. Verein der Aerzte Stettins, October, 1911.

the case of a 16-year-old boy. *B. typhosus* was isolated from the abscess. Intestinal lesions were absent.

*Gall-Bladder.*—Primary typhoidal inflammation of the gall-bladder is a recognized condition. In 1896, Kühnau<sup>29</sup> reported the following case: Female, aged 32 years. Died in the eighth week of the disease. Spleen moderately enlarged. Abscesses in the mesenteric nodes and kidneys. Suppurative cholecystitis. *B. typhosus* from the suppurative foci. Cushing<sup>30</sup> noted a case of gall-bladder infection and calculus formation with no previous history of typhoid fever. He discussed a possible agglutinative reaction in the bile and its relation to calculus formation. A young woman with typhoidal temperature and the symptoms of cholecystitis was seen by Besançon and Philibert.<sup>31</sup> Headache, diarrhea, roseola and splenic tumor were absent. Diazo positive. Widal positive 1:600. No note of bacteriological examination. A patient with suppurative hepatitis, cholecystitis and caval thrombosis was observed by Müller.<sup>32</sup> Widal positive 1:500. Additional case reports have been made by Morris,<sup>33</sup> Legendre,<sup>34</sup> Pratt,<sup>35</sup> Stewart,<sup>36</sup> Wilson,<sup>37</sup> Posselt<sup>38</sup> and Holmes.<sup>39</sup> Quenu and Duval<sup>40</sup> believe that many cases of benign and febrile icterus, Weill's disease, etc., are primary biliary infections with typhoid bacilli. Carlisle and Martin<sup>41</sup> observed a case of cholecystitis with calculi, necrosis and hepatic abscesses, and summarized fifty-two cases of primary gall-bladder infection.

*Kidneys.*—Typhoid fever may present itself with unusual renal symptoms—so-called nephrotypoid. Thue's<sup>42</sup> case of acute hemorrhagic nephritis with ulcers of the colon and anus falls in this group. Numerous masses of typhoid bacilli were found in the renal tissues. Vanzetti<sup>43</sup> noted a 15-year-old girl with severe parenchymatous nephritis and uremia. Necropsy showed renal infarcts in which typhoid bacilli were

29. Kühnau: Berl. klin. Wehnschr., 1896, No. 30, p. 666.

30. Cushing: Bull. Johns Hopkins Hosp., 1898, ix, 91.

31. Besançon and Philibert: Bull. et. mém. Soc. med. d. hôp. de Paris, Nov. 29, 1901, p. 1230; Semaine méd., 1901, p. 396.

32. Müller, R.: Ztschr. f. Heilk., Prague, 1905, xxvi, Abt. f. path. Anat., p. 263.

33. Morris: Typhoid Infection with Primary Focus in the Gall-Bladder, New York Med. Jour., 1899, lxi, 122.

34. Legendre: Bull. et mém. Soc. méd. d. hôp. de Paris, Dec. 6, 1901.

35. Pratt: Typhoid Cholecystitis, with Observations upon Gall-Stone Formation, Am. Jour. Med. Sc., 1901, cxxii, 584.

36. Stewart: A Case of Primary Typhoid Cholecystitis with Calculi, Am. Med., 1904, vii, 1018; Proc. Philadelphia Co. Med. Soc., 1904, xxv, 109.

37. Wilson: Primary Typhoid Cholecystitis and Cholangitis, Jour. Am. Med. Assn., 1908, i, 1607.

38. Posselt: Ergebn. d. allg. Path., 1912, i, 265.

39. Holmes: New York Med. Jour., 1911, xciv, 315.

40. Quénu and Duval: Les angiocholites aiguës, Soc. int. d. chir., 1909.

41. Carlisle and Martin: Proc. Path. Soc., New York, 1908, viii, 138.

42. Thue: Norsk. Mag., 1889, p. 272.

43. Vanzetti: Arch. p. le sc. med., 1902, xxvi, 59.



found. The spleen and intestines were normal. Flexner<sup>44</sup> has reported a case of typhoidal septicemia with focal renal abscesses from which *B. typhosus* was isolated. Bilateral renal abscesses have been reported by Krokiewicz.<sup>45</sup> Napier and Buchanan<sup>46</sup> have noted the persistence of hematuria for five months after infection and a similar case, though of shorter duration, was seen by Lesieur.<sup>47</sup> The bibliography further includes cases published by Kühnau,<sup>48</sup> Nattan-Larrier,<sup>48</sup> Scheib,<sup>49</sup> Monroe and Campbell<sup>19</sup> and Politzer.<sup>50</sup>

*Lungs and Pleurae.*—Typhoid bacilli sometimes produce pulmonary and pleural lesions, chiefly lobar and bronchopneumonia and pleural effusion. Bronchopneumonia appears to be the commonest of these lesions. Flexner<sup>51</sup> noted a case in which thrombosis of the pulmonary artery, pulmonary gangrene, pyopneumothorax and cholelithiasis were found. The intestines showed no evidences of typhoid fever. Examination revealed *B. typhosus* in the lungs, heart's blood, spleen, liver, kidneys and cerebrospinal fluid. Robinson observed a pneumotypoid of the lobar type (cited by McCrae). Among fourteen cases with satisfactory cultures (cited by McCrae), typhoid bacilli in pure culture were isolated in two cases, typhoid and colon bacilli in two cases and these organisms with staphylococci in one case. Rau,<sup>52</sup> Ortner and Glaser<sup>53</sup> observed cases of pneumotypoid with the sputum containing the specific organism. Several other observers have noted this condition. The literature in pneumotypoid includes cases reported by Bruhl,<sup>54</sup> Castaigne,<sup>55</sup> Viola,<sup>56</sup> Grasset,<sup>57</sup> Bensis,<sup>58</sup> Du Cazal,<sup>59</sup> Kogawa,<sup>60</sup> Picchi,<sup>61</sup> Fallet and Bourdin-

44. Flexner: Jour. Pathol. and Bacteriol., 1895, iii, 202; Bull. Johns Hopkins Hosp., iv.

45. Krokiewicz: Typhusbazillen im Blute und Gruber-Widalsche Reaktion bei Pyämie., Wien klin. Wehnschr., 1908, p. 1633.

46. Napier and Buchanan: Glasgow Med. Jour., December, 1906.

47. Lesieur: Fièvre typhoïde à début brusque hématurique, Bull. et mém. Soc. méd. d. hôp. de Paris, Feb. 3, 1911.

48. Nattan-Larrier: Infections éberthienne et streptococcique, Compt. rend. Soc. anat., 1902, p. 827.

49. Scheib: Zur Kenntnis der typhösen Nephritis, Prag. med. Wehnschr., 1902 p. 257.

50. Politzer: Wien. med. Wehnschr., 1912, p. 207.

51. Flexner: Bull. Johns Hopkins Hosp., 1900, viii.

52. Rau: Ztschr. f. Heilk., Prague, 1904, xxv, 385. (Literatur über Pneumotypus.)

53. Glaser: Deutsch. med. Wehnschr., 1902, p. 772. (Literatur.)

54. Bruhl: Gaz. hebdom. de méd., 1897, No. 9.

55. Castaigne: Ibid., Gaz. hebdom. de méd., No. 55.

56. Viola: Gazz. d. osp., 1899, No. 12.

57. Grasset: Rev. intern. de méd. et de chir., Paris, 1906, xvii, 121.

58. Bensis: Grèce méd., 1907, ix, 16.

59. Du Cazal: Bull. et mem. Soc. méd. d. hôp. de Paris, 1893, p. 243.

60. Kogawa: Jikwa Zasshi, Tokio, 1907, p. 248.

61. Picchi: Lo Sperimentale, 1899, 1900, liii, 299.

ière<sup>62</sup> and Bergasse.<sup>63</sup> Fewer examples of typhoidal pleuritis can be found. McNaughton and Rhea,<sup>64</sup> and Charin and Roger,<sup>65</sup> have seen acute hemorrhagic pleuritis; Sahli,<sup>66</sup> a serous pleuritis. Finley<sup>67</sup> summarized 2,200 cases of typhoid fever and found that nineteen presented evidences of pleurisy. He believes that this condition is induced in the majority of cases by the typhoid bacillus and that it is of benign character.

*Brain.*—The literature on meningo- and cerebro-typhoid includes a number of cases. Fernet<sup>68</sup> reported one case with strabismus and exophthalmos, but no diarrhea, roseola, etc. Section showed no splenic enlargement nor intestinal lesions, but *B. typhosus* was isolated from the cerebrospinal fluid. Merkens<sup>69</sup> noted another case in which the patient had been operated on for chronic purulent otitis media and soon afterward developed a temperosphenoidal abscess. There was no previous history of typhoid fever. Widal positive. Operation and death. No intestinal lesions. McCaskey and Porter,<sup>70</sup> Gurd and Nelles,<sup>71</sup> and Melchior<sup>72</sup> have recorded cerebral abscesses in which typhoid bacilli were found. Purulent meningitis cases have been reported by Norman and Rosenburger<sup>73</sup> and Stäubli.<sup>74</sup> Caussade and Phillips<sup>75</sup> have mentioned a case in which a "meningotyphus" was apparently superimposed on an old pachymeningitis. Bergé and Weissenbach,<sup>76</sup> Flexner, Lavenson,<sup>77</sup> and Norman and Rosenburger<sup>73</sup> isolated at autopsy *B. typhosus* from the cerebrospinal fluid. On lumbar puncture Stühmer<sup>78</sup> and Schwartz<sup>79</sup> each

62. Fallet and Bourdinière: Progrès med., 1911, xxvii.

63. Bergasse: Bull. Soc. méd. chir. de la Drôme, 1911, xiii, 162.

64. McNaughton and Rhea: Jour. Can. Med. Assn., Sept. 1912.

65. Charin and Roger: Presence du bacille d'Eberth dans un épanchement pleural hémorrhagique, Bull. et mém. Soc. méd. d. hôp. de Paris, 1891, p. 185.

66. Sahli: Centralbl. f. Bakteriöl., Ref., 1894, xvi, 651.

67. Finley: Jour. Can. Med. Assn., Sept., 1912.

68. Fernet: Bull. et mém. Soc. méd. d. hôp. de Paris, 1891, p. 361.

69. Merkens: Zentralbl. f. Chir., 1898, p. 1058.

70. McCaskey and Porter: A Case of Brain Abscess Due to Latent Typhoid Infection. Operation: Death from Cardiac Complication, Jour. Am. Med. Assn., 1903, xl.

71. Gurd and Nelles: Ann. Surg., 1908, i, 4.

72. Melchior: Centralbl. f. d. Grenzgeb. d. Med. u. Chir., 1911, Nos. 1-2.

73. Norman and Rosenburger: Purulent Cerebrospinal Meningitis Caused by the Typhoid Bacillus, without the Usual Intestinal Lesions of Typhoid, Am. Jour. Med. Sc., 1908, cxxxv, 240; Proc. Path. Soc., Philadelphia, 1908, xi, 52.

74. Stäubli: Deutsch. Arch. f. klin. Med., 1904, lxxxii, 90.

75. Caussade and Phillips: Bull. et mém. Soc. méd. d. hôp. de Paris, 1911, p. 867.

76. Bergé and Weissenbach: Bull. et mém. Soc. méd. d. hôp. de Paris, 1911, No. 30.

77. Lavenson: Typhoid Meningitis without Other Lesions, Univ. Penn. Med. Bull., April, 1908.

78. Stühmer: München. med. Wchnschr., 1910, p. 357.

79. Schwartz: Med. Rec., New York, Oct. 29, 1910.

found the organism in one case. The former summarized the literature of eight cases in which during life the organism had been isolated. Cole<sup>80</sup> has suggested that serous meningitis due to typhoid bacilli is probably more common than is supposed. Further contributions to literature on meningotyphoid have been made by Vincent,<sup>81</sup> Bruno<sup>26</sup> and Picchi.<sup>82</sup>

*Unusual Alimentary and Laryngeal Lesions.*—Aberrant typhoidal ulcers have been found in the mouth, fauces, pharynx, larynx, esophagus, stomach, rectum and at the anus. Louis was the first to call attention to esophageal ulcers in typhoid fever. In Koranyi's clinic, Vas<sup>83</sup> noted in a series of 322 typhoid patients eight with specific pharyngeal and laryngeal lesions. In ten examinations Besson<sup>84</sup> found typhoid bacilli six times in the tonsils. In an epidemic, Cappellari<sup>85</sup> observed five severe cases with characteristic tonsillar ulcers. These lesions have also been bacteriologically studied by Bendix and Bickel.<sup>86</sup> Mollard<sup>87</sup> has reported a case with initial tonsillar involvement; death on the fifteenth day; necropsy showed numerous typical typhoidal ulcers of the intestines. According to Levy and Gaetgens,<sup>88</sup> the portal of entry for the bacilli in some cases is doubtless the tonsils from which lymphatic dissemination occurs and eventually blood and intestinal infection. Kimla saw a unilateral tonsillar ulcer with *B. typhosus* in the necrotic tissues. Troullier<sup>89</sup> has mentioned specific ulcers of the soft palate, tongue, gums and pharynx, and in 220 typhoid patients he noted eighty-one with ulcers of the mouth and pharynx. In thirty-six of fifty-one cases, Manicatide<sup>90</sup> noted these ulcers from which the organism was isolated on Conradi-Drigalski media. Marquardt (cited by McCrea) reported having found ulcers of the pharynx, palate or tonsils in three or four cases at Ebstein's clinic. In a typhoid patient, Novotny<sup>91</sup> isolated the paratyphoid "B" bacillus from a palatal ulcer. While in the third Medical Clinic in Vienna he published statistics on laryngeal ulcers in typhoid fever. He describes them as being smooth, round or oval, sharply defined and shallow, and in the early stage with red margins. The bases are yellow or grayish yellow and without false membrane. On irritation they bleed

80. Cole, cited by McCrae: Osler's Modern Medicine, ii, 157.

81. Vincent: Recherches bacteriologiques sur l'infection mixte par le bacille typhique et le streptocoque, Bull. et mém. Soc. méd. d. hôp. de Paris. 1891, p. 561.

82. Picchi: La clin. méd., 1906, No. 37.

83. Vas: Pest. med.-chir. Presse, 1893, No. 36.

84. Besson: Rev. de med., 1897, No. 6.

85. Cappellari: Gazz. d. osp., 1899, No. 43.

86. Bendix and Bickel: Deutsch. med. Wchnschr., 1907, No. 23.

87. Mollard: Lyon méd., 1906, cvi, 798.

88. Levy and Gaetgens: Arb. a. d. k. Gsndhtsamte, 1908, xxviii, 168.

89. Troullier: Gaz. d. hôp., 1908, No. 18.

90. Manicatide: Centralbl. f. Bakteriöl. (Orig.), 1908, xlvi, 221.

91. Novotny: Wien. klin. Wchnschr., 1909, xxii, 779. (Literatur.)

slightly. Healing takes place without scars. The points of predilection are the anterior pillars and the soft palate. Gallois, Courcoux and Decobert<sup>92</sup> have demonstrated *B. typhosus* in nasal secretions of patients. Clifford and Moore<sup>93</sup> have recently noted a case of bilateral cancrum oris complicating typhoid fever in a patient 8 years old. Recovery. Keen has collected nine such cases from the literature and of these, five were fatal. In a series of necropsy records cited by McCrae in Osler's "Modern Medicine," four out of 2,544 typhoid cases showed esophageal ulcers. *B. typhosus* has been found in such ulcers, but most of them are probably due to simple pyogenic organisms. Two of these showed a diphtheroid esophageal membrane. Stricture is a rare sequel of the condition. Additional recent contributions to the literature on typhoidal lesions of the upper respiratory and alimentary tracts have been made by Blum,<sup>94</sup> Schmidt,<sup>95</sup> Lüdin<sup>96</sup> and Kirchgaesser.<sup>97</sup> A case of perforated typhoidal gastric ulcer has been reported. Chantemesse<sup>98</sup> and Guinard were apparently the first observers to call attention to the existence of typhoidal appendicitis. Posselt, McCrae, Stokes and Amick<sup>99</sup> have contributed to the literature. Posselt,<sup>100</sup> Mussat,<sup>101</sup> Mazeran,<sup>102</sup> Pfister,<sup>103</sup> von Czyhlarz<sup>104</sup> and Ortner<sup>105</sup> have noticed a complicating membranous enteritis. Thue's<sup>42</sup> case showed ulcers at the anus.

*Bone.*—Except for bone lesions developing during the course of typhoid fever these manifestations are not sufficiently unusual to justify their discussion. McCrae (Osler's Modern Medicine) cites a boy, 10 years of age, who during the course of the disease developed fluctuating masses on the frontal bone and on one rib. *B. typhosus* was not isolated from the pus.

*Skin.*—The more unusual cutaneous manifestations are herpes, urticaria, erythema, erysipelas, desquamation, gangrene, purpura, impetigo and striae atrophicae. McCrae has reported twenty cases of herpes in a

92. Gallois, Courcoux and Decobert: Rhino-pharyngite typhoïdique à bacilles d'Eberth, Bull. et mém. Soc. méd. d. hôp., de Paris, Nov. 28, 1902.

93. Clifford and Moore: Jour. Am. Med. Assn., 1912, lviii, 1145.

94. Blum: Semaine méd., 1908, p. 37.

95. Schmidt: Ztschr. f. path. Anat., 1907, No. 15.

96. Lüdin: Ueber den diagnostischen Wert der typhösen Gaumenschwüre, Corr.-Bl. f. Schweizer Aerzte, 1910, xl, 751.

97. Kirchgaesser: München. med. Wehnschr., 1911, No. 23.

98. Chantemesse: Traité de méd.: Fièvre typhoïde.

99. Stokes and Amick: Typhoid Appendicitis without Other Intestinal Lesions, Bull. Johns Hopkins Hosp., 1905, xvi, 284.

100. Posselt: Ergebn. d. alleg. Path., 1912, i, 323.

101. Mussat: Bull. Soc. méd. de Reims, 1899, v, 354.

102. Mazeran: Jour. de med. de Paris, 1906, xviii, 112; Lyon méd., 1908, No. 30.

103. Pfister: Deutsch. med. Wehnschr., 1906, p. 1285.

104. von Czyhlarz: Arch. f. Verdauungskr., 1910, xvi, 576.

105. Ortner: Arch. f. Verdauungskr., 1910, xvi, 726.



series of 1,500 typhoid cases. Zinn<sup>106</sup> found them in ten of 190 cases. Among others, Krokiewicz and Wenzke have published case reports. Urticaria was observed by Miller<sup>107</sup> in two out of 150 cases. Murchison<sup>108</sup> was the first to call attention to the similarity of erythematous eruptions in typhoid fever and scarlet fever. Hutinel,<sup>109</sup> Parker<sup>110</sup> and Richon-Hanns<sup>111</sup> have reported cases. Some no doubt are combined infections, such as have been observed by Gayton,<sup>112</sup> Caiger,<sup>113</sup> Payne,<sup>114</sup> Griffiths,<sup>115</sup> Sequeira,<sup>116</sup> Cosgrove,<sup>117</sup> Coombs,<sup>118</sup> Gabe<sup>119</sup> and Tissot.<sup>120</sup> Erythema nodosum was noted by Osler.<sup>121</sup> Singer<sup>122</sup> has observed an acne-like eruption, Eggleston<sup>123</sup> a pustular and Higgins<sup>124</sup> and Osler<sup>121</sup> a pemphigoid eruption. The coexistence of measles and typhoid has been observed by Ringer,<sup>125</sup> Ringwood,<sup>126</sup> Chrystie,<sup>127</sup> Matiegka<sup>128</sup> and Da Costa. Rolleston<sup>129</sup> saw a 10-year-old boy, who, on the fourteenth day of typhoid fever, showed desquamation over the thorax, abdomen, thighs, heels and toes, some of the desquamated pieces being the size of a shilling. A case of exfoliative dermatitis has been reported by Diehl.<sup>130</sup> Some of the blebs were as large as a half dollar. Appiani<sup>131</sup> observed several cases of general desquamation and summarized the literature. Simmons,<sup>132</sup> Nicholls,<sup>133</sup> Hughes and Levy,<sup>134</sup> McCrae, Liebermeister, Uskins, Cursch-

106. Zinn: München. med. Wehnschr., 1895, xlii, 486.

107. Miller: Internat. Clin., iii, 60.

108. Murchison: Treatise on Continued Fevers in Great Britain, Ed. 2, 1873, p. 515.

109. Hutinel: Arch. gén. de méd., September, 1892.

110. Parker: Bull. Johns Hopkins Hosp., 1911, p. 79.

111. Richon-Hanns: Progrès méd., 1911, xxii, 387.

112. Gayton: Lancet, London, May 19, 1894.

113. Caiger: Lancet, London, 1894, i, 1137.

114. Payne: Lancet, London, 1894, p. 1264.

115. Griffiths: Lancet, London, 1893, ii, 1307.

116. Sequeira: Brit. Med. Jour., 1891, i, 586.

117. Cosgrove: Brit. Med. Jour., 1897, i, 29.

118. Coombs: Brit. Med. Jour., 1897, i.

119. Gabe: Brit. Med. Jour., 1894, p. 848.

120. Tissot: Progrès méd., 1909, p. 230.

121. Osler: Studies in Typhoid Fever, Johns Hopkins Hosp. Rep., p. 473.

122. Singer: Ueber Varietäten des Typhusexanthems und ihre Bedeutung, Wien. klin. Wehnschr., 1896, No. 15.

123. Eggleston: New York Med. Jour., 1910, p. 508.

124. Higgins: Bull. Johns Hopkins Hosp., 1910, xxi, 289.

125. Ringer: Lancet, London, June 30, 1889.

126. Ringwood: Lancet, London, July 7, 1889.

127. Chrystie: Univ. Med. Mag., December, 1888.

128. Matiegka: Prag. med. Wehnschr., Sept. 25, 1889.

129. Rolleston: Tr. Clin. Soc., London, 1890, xxiii, 84.

130. Diehl: Jour. Cutan. Dis., 1898, xvi, 222.

131. Appiani: Gazz. d. osp., 1907, No. 12.

132. Simmons: Delaware State Med. Jour., September, 1912.

133. Nicholls: Montreal Med. Jour., June, 1906.

134. Hughes and Levy: Arch. de méd. et de phar., August, 1892; quoted by Hare and Beardsley, Typhoid Fever and Exanthemata, Ed. 2, 1909, p. 179.

mann, Fiessinger and Weil<sup>135</sup> have written on hemorrhagic syndromes ("hemorrhagic putrid typhoid"—French) early and late in typhoid fever. Gangrene of the skin has been noted by Taupin,<sup>136</sup> Hare and Beardsley,<sup>137</sup> Jacobi<sup>138</sup> and Abt.<sup>139</sup> A Series of such cases has been described by McFarland<sup>140</sup> and Stahl.<sup>141</sup> Erysipelas was found by Liebermeister<sup>142</sup> in ten of 1,420 typhoid cases; in ten of 500 cases, by Griesinger.<sup>143</sup> Among others, Freudenberger,<sup>144</sup> Potain,<sup>145</sup> Thielmann,<sup>146</sup> Berthoud,<sup>147</sup> Martine,<sup>148</sup> Armieux,<sup>149</sup> and Hare and Beardsley<sup>137</sup> have reported cases of erysipelas. Wilks<sup>150</sup> was the first observer to describe patellar striae. Bradshaw,<sup>151</sup> Shepherd,<sup>152</sup> Troisier,<sup>153</sup> Northrup,<sup>154</sup> Fischer,<sup>155</sup> Köbner,<sup>156</sup> Duckworth,<sup>157</sup> Kaposi,<sup>158</sup> Gubler,<sup>159</sup> Bouchard,<sup>160</sup> Nonne,<sup>161</sup> Barie,<sup>162</sup> Langer,<sup>163</sup> and Bunch<sup>164</sup> have noted the condition. The most frequent site of these lesions is the patellar region and more rarely over the anterior surface of the lower third of the leg, over the abdomen and over the arms and fingers. Phillips<sup>165</sup> has summarized 1,230 typhoid cases from the Lakeside Hospital, Cleveland. Herpes were seen in 12 cases; urticaria in 21 cases; desquamation in 83 cases; erythema in 7 cases;

135. Fiessinger and Weil: *Rev. de med.*, xxxii. No. 9.

136. Taupin: *Jour. d. Con. méd.-chir.*, 1839, No. 7.

137. Hare and Beardsley: *Typhoid Fever and Exanthemata*, Ed. 2. 1909, pp. 177. 253.

138. Jacobi: *Arch. Pediat.*, Dec. 15. 1899.

139. Abt: *Jour. Am. Med. Assn.*, 1901, xxxvii. 445.

140. McFarland: Quoted by Hare and Beardsley, p. 179. (Note 134.)

141. Stahl: Quoted by Hare and Beardsley, p. 178. (Note 134.)

142. Liebermeister: Quoted by Hare and Beardsley, p. 253. (Note 134.)

143. Griesinger: Quoted by Curschmann in Nothnagel's *Encyclopedia*, Typhoid and Typhus Fevers, p. 325.

144. Freudenberger: Quoted by Hare and Beardsley, p. 258.

145. Potain: Quoted by Hare and Beardsley, p. 259.

146. Thielmann: Quoted by Hare and Beardsley, p. 257.

147. Berthoud: Quoted by Hare and Beardsley, p. 258.

148. Martine: Quoted by Hare and Beardsley.

149. Armieux: Quoted by Hare and Beardsley, p. 257.

150. Wilks: *Guy's Hos. Rep.*, 1861, vii.

151. Bradshaw: *Liverpool Med.-Chir. Jour.*, July, 1888.

152. Shepherd: *Tr. Am. Dermat. Assn.*, 1890, xiv, 23.

153. Troisier: *Bull. et mém. Soc. méd. d. hôp. de Paris*, 1889, No. 12.

154. Northrup: *Tr. Assn. Am. Phys.*, 1903.

155. Fischer: *München, med. Wehnschr.*, 1904, p. 482.

156. Köbner: *München, med. Wehnschr.*, 1904, p. 928.

157. Duckworth: *Brit. Jour. Dermat.*, 1893, p. 357.

158. Kaposi: *Diseases of the Skin*, Hebra, iii, 262.

159. Gubler: Quoted by Bunch, *Brit. Jour. Dermat.*, 1905, xvii.

160. Bouchard: Quoted by Bunch, *Brit. Jour. Dermat.*, 1905, xvii.

161. Nonne: Quoted by Bunch, *Brit. Jour. Dermat.*, 1905, xvii.

162. Barie: *Semaine méd.*, 1888, p. 259.

163. Langer: *Anzeig. d. k. k. Gesell. d. Aerzte.*, Wien., 1879, No. 28.

164. Bunch: *Brit. Jour. Dermat.*, 1905, xvii.

165. Phillips: *Skin Rashes in Typhoid Fever*, *Am. Jour. Med. Sc.*, Aug., 1910.

purpura in 6 cases; pemphigoid eruption in 4 cases; erysipelas in 2 cases; impetigo in 1 case; striae patellares, in 1 case. In the last mentioned there were 42 transverse striations over the patellae and lower third of the legs.

#### DUAL INFECTIONS

The coexistence of miliary tuberculosis and typhoid fever was observed by Meunier<sup>166</sup> in an 8-year-old boy in whom a generalized miliary tuberculosis was present without evident typhoidal lesions; nevertheless *B. typhosus*, as well as *B. tuberculosis*, was isolated from the spleen, pleural fluid and lungs. Griffon<sup>18</sup> has reported a case of mixed infection with a streptococcus isolated from the bronchial pus, spleen, gall-bladder and bone-marrow together with *B. typhosus* from these organs. A case of endocarditis was reported by Nattan-Larrier,<sup>48</sup> who isolated a streptococcus and *B. typhosus* from the heart's blood and spleen, and the former from the miliary abscesses in the kidneys. Vincent<sup>167</sup> isolated the same types of organisms from the spleen, liver, kidneys and heart's blood in a case without intestinal lesions. Typhoid fever has coexisted with various other infections, such as diphtheria, scarlet fever (Fournier), measles, malaria, small-pox, chicken-pox, whooping-cough, etc.

#### INFANCY AND CHILDHOOD

Typhoid fever in early life, according to some writers, is likely to assume a septicemic form and to present no typical intestinal lesions. The disease is certainly more common at this period of life than was once supposed. There appears to be a tendency toward localization of lesions in the lungs and central nervous system. Griffith and Ostheimer<sup>168</sup> have reported on twelve congenital cases. Sacquépée and Chevre<sup>169</sup> noted two cases in patients 3 and 4 years old, without involvement of the bowel, with agglutinations 1:200 and 1:300 and with *B. typhosus* from the heart's blood. A 16-months-old child with suppuration of both parotids was observed by Goldberger.<sup>170</sup> Halbron and Nandrot<sup>171</sup> have reported a case of a 5-year-old boy with ambulant typhoid and perforation. Barrett<sup>172</sup> observed a 2-year-old child with intestinal hemorrhage and remittent fever, but no roseola nor splenic enlargement. There were symptoms of meningitis. Additional cases of typhoidal septicemia without intestinal involvement have been reported by Cheadle, Bryant, Guiz-

166. Meunier: Bull. et mém. Soc. méd. d. hôp. de Paris, 1897, p. 476; Semaine méd., 1897, p. 121.

167. Vincent: Ann. de l'Inst. Pasteur, 1893, vii, 141.

168. Griffith and Ostheimer: Quoted by McCrae, Osler's Modern Medicine, ii, 176.

169. Sacquépée and Chevre: Progrès méd., 1909, p. 73.

170. Goldberger: Aeztl. Ztrl.-Anz. Wien., 1896, viii, 209.

171. Halbron and Nandrot: Tribune méd., Paris, Oct. 14, 1905.

172. Barrett: Med. Rec., New York, lxxx, No. 2145.

zetti, Opie and Bassett, Lazarus-Barlow,<sup>173</sup> Lucksch,<sup>174</sup> Ribadeau-Dumas and Boyé,<sup>175</sup> and Schottmüller.<sup>176</sup>

#### TRAUMATISM

This has been suggested as an important predisposing factor in typhoidal suppurations. Seiler<sup>177</sup> has presented a dissertation on post-traumatic typhoidal peritonitis and Sennert,<sup>178</sup> on liver abscess as a sequel of trauma. The case of Sergent and Bernard<sup>179</sup> developed a typhoidal meningitis, according to the observers, after a bullet wound in the head. Gurd and Nelles<sup>71</sup> reported the case of cerebral abscess presumably developing in consequence of a head injury. The abscess was said to be of typhoidal character. Barjon and Lesieur<sup>180</sup> have presented a case of "arthrotyphus" succeeding "rheumatism," when trauma possibly played some part.

#### AGGLUTINATION REACTIONS

At times a moderately strong typhoidal agglutinating blood-serum is observed when the individual obviously presents no clinical evidence of typhoid fever. One of five possibilities may exist: (1) an infection with some other organism of the typhoid-colon-dysentery group; (2) a typhoidal infection of "extra-intestinal type"; (3) a recent typhoidal infection; (4) a recent prophylactic vaccination; (5) "typhoid carrier." Positive serum agglutinations with typhoid bacilli have been reported in miliary tuberculosis, pneumonia, sepsis, etc., but a previously existing typhoidal infection probably accounts for most, if not all, of these. Symmers and Wilson<sup>181</sup> have reported a case of epidemic cerebrospinal meningitis having a Widal positive 1:200. There was no previous history of typhoid fever. Necropsy showed no intestinal involvement and bacteriological examination of spleen, mesenteric nodes and urine was negative. Meningococci in pure culture isolated from meninges. Stelker<sup>182</sup> reported another epidemic meningitis case with a Widal positive 1:100. Becker and Ruhland<sup>183</sup> have made further reports.

173. Lazarus-Barlow: *Brit. Med. Jour.*, 1901, p. 792.

174. Lucksch: *Centralbl. f. Bakteriöl. (Orig.)*, 1903, xxxiv, 113.

175. Ribadeau-Dumas and Boyé: *Arch. d. méd. d. enf.*, 1909, xii, 584; *Jahrb. f. Kinderh.*, 1910, p. 105.

176. Schottmüller: *München. med. Wehnschr.*, 1911, p. 469.

177. Seiler: *Posttraumatische Peritonitis typhosa bei bestehender typhöser Cholezystitis*, Inaug.-Dissert., Berlin, Nov., 1910.

178. Sennert: *Inaug.-Dissert.*, Halle, July, 1906.

179. Sergent and Bernard: *Bull. et mém. Soc. méd. d. hôp. de Paris*, 1901, p. 57.

180. Barjon and Lesieur: *Province méd.*, Oct. 27, 1900; *Jour. d. Phys. et Path. gén.*, 1901, p. 150.

181. Symmers and Wilson: *Brit. Med. Jour.*, 1907, p. 713.

182. Stelker: *Russk. Vrach.*, 1907, No. 8.

183. Becker and Ruhland: *Jour. Am. Med. Assn.*, 1909, lii, 13.



## THROMBOPHLEBITIS

Though not an unusual lesion of typhoid fever, thrombophlebitis bears mention in this place. One of the most recent and exhaustive summaries of this condition has been published by Connor<sup>184</sup> in a study of eighty-two cases. He states that the condition is more common than is supposed (10 to 15 per cent. of all cases of typhoid fever), that it is gradual in development and that it is more extensive than the symptoms would indicate. He believes that most pulmonary and pleural complications late in typhoid fever are due to embolism of the pulmonary artery, that obscure, late, recurring chills are regularly associated with venous thrombosis, and that "tender toes" in the majority of cases are caused by thrombophlebitis.

Lesions of the intestines, mesenteric nodes and spleen were absent in those cases reported by Lazarus-Barlow, Pick,<sup>185</sup> Picchi, Barjon and Lesieur and Vanzetti. Unusual splenic enlargement was noted by Nicholls and Keenan,<sup>186</sup> Bozzolo,<sup>187</sup> Karlinski,<sup>21</sup> Doglietti,<sup>16</sup> Guizzetti<sup>23</sup> and Blumenthal.<sup>17</sup> An unusually small spleen (one-half normal) was reported by Roscoe.<sup>188</sup> Monroe and Campbell's<sup>19</sup> case showed perisplenic abscess; Picchi's and Blumenthal's cases, renal infarcts. In the cases without intestinal lesions, "pea-soup stools" were observed by Hodenpyl,<sup>189</sup> Blumenthal, Jores<sup>190</sup> and Posselt. In the same class of cases, *B. typhosus* was isolated from the stools by Hodenpyl, Pick, Bobbio, Gennari,<sup>191</sup> Farey,<sup>192</sup> Etienne and Thiry. This organism was isolated from the mesenteric nodes by Lartigau (also *B. coli*), Bryant, Nicholls and Keenan, Banti,<sup>193</sup> Chiari<sup>194</sup> and Vestenrik.<sup>195</sup>

A review of the literature reveals a large number of cases classified as atypically typhoid, but in many of these the diagnosis of lesions was not established by bacteriological examination. Many cases have been published as primary typhoidal gall-bladder infections in which it seems probable that a previously existing infection left the patients as "carriers." Nevertheless, many writers, among whom are McCrae, Phillips, Luksch, Chiari, Brion, Keyser and Posselt have laid emphasis on the

184. Connor: THE ARCHIVES INT. MED., 1912, x, 534.

185. Pick: Wien. klin. Wehnschr., 1897, p. 82.

186. Nicholls and Keenan: Montreal Med. Jour., 1898, xxvii, 9.

187. Bozzolo: Tenth Internat. Med. Congress, Berlin, 1890, ii, 188; Wien. med. Wehnschr., 1890, p. 226.

188. Roscoe: Lancet, London, Oct. 16, 1909.

189. Hodenpyl: Brit. Med. Jour., 1897, p. 1850.

190. Jores: München. med. Wehnschr., 1911, p. 1247.

191. Gennari: Gazz. d. osp., 1907.

192. Farey: Thèse de Paris, 1909, No. 267.

193. Banti: Riforma med., 1894, x, 674.

194. Chiari: Ztschr. f. Heilk., Prague, 1897, xviii, 471.

195. Vestenrik: Soc. d. microb. de Saint Petersburg, 1904.

fact that typhoid infection without intestinal lesions is a very rare condition. In twenty years at the Institute of Pathological Anatomy at Innsbruck, von Hibler failed to note a case. In 1899, Howard<sup>9</sup> collected from the literature 164 cases of typhoidal infection (including mixed infection) without intestinal localization. During the same year McPhedran<sup>196</sup> published a summary of twenty cases. In 1912, in Lubarsch and Ostertag's "Ergebnisse der allgemeinen Pathologie," Posselt presented an admirable review of the literature, and states that only 63 cases fulfill the requirements of accurate bacteriological examinations.

Primary typhoidal septicemia .....	28
(Mixed infection, including tuberculosis) .....	7
Primary typhoidal meningitis .....	3
Primary typhoidal affections of the lungs .....	2
Primary typhoidal affections of the pleurae .....	1
Primary typhoidal affections of the spleen .....	3
Primary typhoidal affections of the kidneys .....	2
Primary typhoidal affections of the joints .....	1
Primary typhoidal affections of the liver and bile ducts...	16
	<hr/>
	63

On reviewing the reports it is quite evident to us that the literature contains many incomplete and inaccurate observations, but the above table evidently does not include a number of authentic reports.

As to the nomenclature, the German and French writers have made much use of the terms, meningo-typhoid ("meningotyphus"), pneumo-typhoid ("pneumotyphus"), etc. It has been suggested that the terms, as indicated in the above table, should be employed. However, the foreign terms have been employed with broader significance so as to imply not necessarily a primary lesion without intestinal involvement, but to emphasize in general a dominant or unusual lesion in a case otherwise presenting the classical evidences of typhoid fever.

#### SUMMARY OF CASES

The following represents a summary of cases at the Southern Pacific Hospital, San Francisco, and others seen in private practice and consultation, illustrating atypical conditions and complications. Among 149 patients treated at the new hospital since its opening in September, 1909, there have been 11 deaths—7.3 per cent.; with meningitis, chronic nephritis and purulent appendicitis, each 1 case; intestinal hemorrhage, 2 cases; perforation and possibly embolism, each 3 cases. There were 3 paratyphoid cases. Relapses developed in 9 cases, of which one appeared as late as four weeks after leaving the hospital. The longest febrile period of relapse was thirty days and the shortest, nine days. One patient devel-

196. McPhedran: Can. Jour. of Med. and Surg., Toronto, 1899, vi. 251; Philadelphia Med. Jour., 1900, i. 543.

oped a second attack of typhoid fever after a six-year interval. The earliest intestinal hemorrhage occurred on the third day. Two cases were fatal and of 11 others, 4 were severe and 7 were mild. Pleurisy with effusion (organism?), meningitis and pneumonia (*B. typhosus*), neuritis in leg, neuritis in shoulder, periostitis of tenth rib and perforating duodenal ulcer were each noted in one case. The ulcer patient was operated on on the twenty-ninth day, but the lesion was not determined as typhoidal. Two patients presented an initial appendicitis, the operation being followed by the development of typhoid fever. Another patient during the course of typhoid infection developed the symptoms of perforation; operation was performed and a ruptured appendix was disclosed. Phlebitis of the leg developed in 7 cases, and of these there were 4 in the second week, 2 in the fifth week and 1 in the third week after discharge. Thrombosis or embolism was suspected in the lungs in 4 cases and in the mesenteric veins in 3 cases. These occurred from the sixth to the thirty-fifth days, and were not severe, except in 2 cases of pulmonary embolism. One patient died promptly, and the other, occurring on the thirtieth day, resulted in gangrene from which the patient was convalescent on the one hundred and ninety-first day. Chills and fever were noted in 13 cases of which phlebitis of the leg was the etiological factor in 3. Malaria may have been a factor in another case. Chills occurred during the first week in 4 cases, second week in 2 cases, third week in 4 cases, fourth week in 2 cases and sixth week in 1 case.

In California and Nevada, 47 cities and towns furnished typhoid cases. San Francisco, 17; Sacramento, 14; Oakland and Alameda, 13; Bakersfield, 5; Sparks (Nevada), 25.

Additional cases are quoted in brief to illustrate certain of the complications, two cases of hemorrhagic form being especially uncommon.

*Typhoidal Cholecystitis*.—F. F., male, aged 40 years, had an afebrile period of one week following typhoid fever. Then developed chilly sensations, epigastric pain, muscular spasm, tenderness in right hypochondrium, slight jaundice, nausea and vomiting. Leukocytes, 6,000; neutrophils, 50 per cent. Within a few days distinct tumor of the gall-bladder elicited, but this had subsided at the end of a week. Highest temperature 103 F. Duration of attack eighteen days. Three years later there had been no further gall-bladder symptoms.

*Thrombosis of Mesenteric Vessels*.—V. J. D. C., male, aged 40 years, was seen in the fifth week of typhoid fever. His general condition had been excellent throughout the course of the disease. During the last ten days there had been successive crops of "rose spots" over the abdomen and back. On examination the abdomen was found to be tympanitic and there was especial distention in the right lower quadrant. Tenderness had been present in this region for some time, according to the previous examiners. There was no dullness in the flanks. Spleen reached to the costal margin. Diagnosis.—Probable thrombosis of mesenteric vessels though condition of relapse was considered. Perforation and complicating appendicitis were eliminated.

*Venous Thrombosis*.—R. E. W., male, aged 20 years. A probable relapse was considered. The usual course of typhoid infection was succeeded by an afebrile

period of seven days. Rise of temperature then developed, lasting four days, during which time he was seen by one of us (P. K. B.). Blood count indicated a late typhoidal condition and no pyogenic complication. A probable relapse was considered. Temperature fell and remained normal for seven days, throughout which time he had a profuse diarrhea, most severe the last two days. Then a sudden rise of temperature to 104 F. appeared, followed by collapse (96 F.). The pupils were widely dilated, extremities cold and clammy, and there were profuse sweats, very feeble low tension pulse of 80 to 90, respirations 20, no evidence of perforation. Strychnin and salt solution were administered with good results. During the next five days he had six similar collapses, often sudden in onset. Pulse did not become rapid, but extremely slow and feeble. Respiration shallow. No unconsciousness. Intravenous or subcutaneous salt solution injections were most effectual. No especial abdominal tenderness. Diagnosis.—Probable thrombosis.

*Hemorrhagic Typhoid.*—(1) C. B. E., male, aged 21 years. "Rose spots" appeared on the sixth day and positive Widal on the seventh. Several intestinal hemorrhages on the ninth day with hematuria and generalized purpura. On the third day bleeding from nose and gums. Death on the twenty-eighth day.

(2) J. M. G., male, aged 36 years. Seen in the third week of typhoid fever. Widal positive on the tenth day. Usual course during first two weeks. On the sixteenth day a large number of small subcutaneous hemorrhages over back from transverse process of scapula to level of sacrum. Small crop covered upper part of buttocks. During the third week patient had hemorrhage from nose, stomach and intestines. Pulse 104 to 120. Very restless. Calcium salts administered per rectum in small doses. Died the following morning.

To the subject in hand no words are more fitting than those of Murchison relative to typhoid fever: "There is no disease, in fact, which exhibits a more protean character, from predominance of certain symptoms, and from the presence of complications."



## THE ABSORPTION OF PROTEIN WITHOUT DIGESTION \*

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The purpose of the experiments reported in this paper is to determine whether it is possible for a protein to be absorbed through the epithelium of the gastro-intestinal tract and appear in an unaltered form in the circulating blood. This question is not a new one in the physiology of digestion. It has been studied by many workers who have employed a variety of experimental methods. Most of the experiments now quoted in the literature were made before the biological specificity of protein was recognized. It is obviously impossible to distinguish sharply between closely allied animal proteins by purely chemical reactions, such as the measurement of the content of nitrogen, phosphorus, sulphur, or by means of the salting-out methods. The newer biological methods, such as the precipitin and anaphylactic reactions, have a high degree of biological specificity, and enable one to distinguish sharply between proteins so closely related as the blood-serum proteins of the horse, beef and sheep, and enable one to distinguish each protein in the presence of others. It is now possible, therefore, by means of these reactions to trace a protein through the intestinal tract into the circulation, to identify it there as an unchanged protein, to determine the length of time it remains in the circulation, and after its excretion by the kidney to identify it in the urine. Because the anaphylactic reaction is so sensitive, and because a positive reaction can be so sharply distinguished from a doubtful or negative reaction, it has been used almost exclusively in the experiments which are herein reported.

When the protein-splitting action of the digestive ferments was first discovered it was thought that the primary cleavage products, proteoses and peptones, were the form in which protein entered the circulation. These substances are soluble and diffusible, and because they may pass through parchment membrane they were believed to be absorbed in this form into the circulation. Further experiments showed, however, that these substances when introduced directly into the blood were highly toxic,<sup>1</sup> and examination of the blood-serum after a heavy protein meal did not show an increase in the proteoses.<sup>2</sup> In fact, recent experiments

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\* Submitted for publication July 8, 1913.

1. Underhill: *Am. Jour. Physiol.*, 1903, ix, 345.

2. Howell: *Am. Jour. Physiol.*, 1906, xvii, 273.

show "proteoses or peptones are not found in the blood, even in the portal blood, collected during the period of maximal absorption from the intestines."<sup>2</sup>

It must be remembered that the blood-serum proteins have a biological specificity; their character does not appreciably change with the character of the diet. Regardless of whether the protein in the food is vegetable or animal in character, the blood-serum proteins have a constant composition. The more recent investigations of the cleavage action of the digestive enzymes indicate that the splitting normally proceeds much farther than was originally believed, and results in the formation of a variety of amino-acids from which protein may be synthesized either by the absorbing epithelium or by other tissues, possibly the liver, after their entrance into the circulation. In fact, there is conclusive evidence that amino-acids are constantly present in the circulating blood.<sup>2</sup> It is believed, therefore, by the majority of physiologists at present that the protein taken in the food is reduced by the process of digestion to the small constituent amino-acids, the so-called "bausteine," which are absorbed as such and resynthesized into protein, either by the intestinal epithelium before reaching the circulation, or by other tissues after the absorption is completed.<sup>3</sup> However, further recent experiments indicate that not all of the amino-acids so formed are used at once for protein synthesis in the manner described.<sup>4</sup> It is well known that the majority of animals take more protein in the food than is required to replace the waste in the tissues. Nitrogen equilibrium may be maintained at a much lower level than the protein intake ordinarily provides. It is believed that a considerable proportion of the amino-acids absorbed after a protein meal are broken down still further, and quickly eliminated, giving rise to the increased nitrogen output which promptly follows nitrogen consumption. In this theory there is no place for the assumption that soluble protein may be absorbed directly without this preliminary cleavage. It must not be overlooked, however, in the light of recent knowledge, that comparatively insignificant amounts of foreign protein may give rise to disturbing and serious manifestations if it gains access to the circulation in an unaltered condition. The amount of nitrogen which, in the form of an alien protein, may cause toxic reactions menacing the life of the animal is so small as to be beyond discovery by the methods commonly followed in measuring the energy exchange.

It was formerly believed that a parenterally introduced protein could not be utilized in the processes of nutrition either as a source of nitrogen or of energy. It was supposed that such protein was immediately

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3. Hammersten-Mandel Text-Book, 1909, p. 412. Abderhalden Text-Book, 1908, pp. 119 and 532.

4. Folin, Otto: *Am. Jour. Physiol.*, 1905, xiii, 117.

excreted by the kidney. New light has been turned recently on this problem by a number of investigators who have demonstrated that the older ideas are incorrect, and that a considerable portion of such protein, the percentage varying with the character of the protein introduced, may be utilized.<sup>6, 7</sup> One may not conclude, therefore, that because no gross quantity of foreign protein appears in the urine after it has been taken as food that none of it has been absorbed as unchanged protein directly into the circulation.

The evidence now quoted in the literature to substantiate the claim that protein may be absorbed without digestion is with few exceptions based on two lines of argument, neither of which gives a positive demonstration that the same identical protein which is introduced into the intestine finally appears in the circulation.

1. The first of these arguments is based on the fact that parenterally introduced protein is not all excreted as such, but is available in some degree for the nutritive requirements of the organism.

2. The second is based on the fact that if certain proteins be introduced into a loop of the intestine or into the colon by rectum and allowed to remain there for varying lengths of time, not all of such protein can be recovered by thoroughly washing out the experimental loop.

The first method of reasoning was followed by Munk and Lewandowsky,<sup>6</sup> who injected casein, egg albumin, acid and alkali albuminates, nucleoproteins and gelatin directly into the circulation and found that not all of the protein so introduced was excreted in the urine. As one conclusion of these experiments they claim to be the first investigators to demonstrate that protein enters unchanged from the intestinal canal directly into the circulating blood. Such a conclusion from such evidence is obviously pure assumption. Numerous other experiments have confirmed their findings with respect to the partial utilization of parenterally introduced protein, but, obviously, such evidence does not prove that protein passes through gastro-intestinal epithelium unaltered.

The second method of experimentation has been employed by many investigators.<sup>7</sup> The evidence which they offer proves beyond doubt that native protein may disappear from a section of the intestine even though digestive enzymes cannot be demonstrated to have any part in the process. It is assumed that because proteins disappear and because no protein-

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6. Munk and Lewandowsky: *Arch. f. Anat. u. Physiol., Suppl. Vol.*, 1899, p. 73; Cramer: *Brit. Jour. Physiol.*, 1909, xxxvii, 146; Oppenheimer: *Hofmeister's Beitr. z. Chem. Phys. u. Path.*, 1904, iv, 263; Heilner: *Ztschr. f. Biol.*, 1907, i, 26.

7. Friedländer: *Ztschr. f. Biol.*, 1897, xxxiii, 264; Czerney and Latschenberger: *Virchows Arch. f. path. Anat.* 1875, lix, 161; Eichherst: *Pflügers Arch. f. d. ges. Physiol.*, 1873, iv, 570; Voit and Bauer: *Ztschr. f. Biol.*, 1871, v, 536; Mendel and Rockwood: *Am. Jour. Physiol.*, 1904, xii, 336.

splitting enzymes can be demonstrated in the loop, that the protein has been absorbed unchanged. This is an obvious conclusion, but one not satisfactorily demonstrated.

Probably the first attempt to make a direct demonstration that unaltered alien protein may appear in the blood after its introduction into the gastro-intestinal tract was that of Ascoli and Vigno,<sup>8</sup> who employed the precipitin reaction as a method of identification. These men experimented entirely with dogs. They collected lymph from the thoracic duct and blood from the jugular vein after egg white and coagulated fowl meat had been introduced into the stomach through a tube. They found that the serum from such animals did not give uniformly constant precipitin reactions with the immune serum; but their reactions were sufficiently constant and sharp to force them to the conclusion that the foreign protein may be absorbed through the stomach either in its native condition or so little modified as not to interfere with its biological reactions. These experiments have been widely quoted in support of the view that undigested protein may pass through the intestinal epithelium unchanged, but they leave the matter still in doubt as to whether the native protein itself or one of its primary cleavage products was the substance actually identified in the blood.

The accuracy and reliability of the experiments herein reported depend on the anaphylactic reaction as a suitable method for identifying the protein. The studies of Wells<sup>9</sup> and Wells and Osborne<sup>10</sup> afford particularly convincing evidence of the suitability of this reaction for this purpose. In a recent paper they have summarized the information as to the specificity of the anaphylactic reaction, and Wells has determined to what extent the processes of digestion so alter a protein as to destroy its specific character as an antigen. It does not seem necessary, in view of the excellent summary which is given in their paper,<sup>10</sup> to review this matter in detail. The earlier conclusions of Rosenau and Anderson<sup>11</sup> in regard to this matter have not been materially modified by recent work. They summarize their conclusions with respect to the specificity of the anaphylactic reaction as follows:

The anaphylactic reaction in the guinea-pig, therefore, seems to be specific in the sense that the precipitins are specific. That is, there is a group reaction in the proteins of allied species, but no reaction between the proteins of widely different species or between proteins of widely different origin.

The testimony of all investigators is agreed that, with few exceptions, notably the protein from the crystalline lens, there is a species specificity

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8. Ascoli and Vigno: *Ztschr. f. physiol. Chem.*, 1904, xxxix, 283.

9. Wells, H. Gideon: *Jour. Infect. Dis.*, 1909, vi, 506; 1908, v, 449.

10. Wells and Osborne: *Jour. Infect. Dis.*, 1911, viii, 66.

11. Rosenau and Anderson: *Jour. Infect. Dis.*, 1908, iv, 552.



to protein which may be sharply identified by means of the anaphylactic reaction. Wells<sup>12</sup> has carried the matter somewhat further in particular cases, and has demonstrated that within a given species there is a specificity which enables one sharply to distinguish proteins. For instance, in a recent work he has demonstrated that:

In hen egg-white there are at least four antigens (three in the coagulable protein and one non-coagulable) which can be distinguished by the anaphylaxis reaction, and therefore are biologically distinct from one another, although coming from a single secretion. At least one other protein of the egg, the ovovitellin, can be differentiated from the other three egg antigens, so that we have here demonstrated five antigens in the egg which are biologically distinct in spite of a common origin. These must be looked on as examples of chemical specificity independent of species specificity. The antigens distinguished by the anaphylaxis reaction seem to correspond to the proteins which have been distinguished by chemical means.

Besides our own experiments, those of many others confirm the principle originally discovered by Rosenau and Anderson that guinea-pigs may be sensitized to a number of proteins at the same time and react to each separately. Rosenau and Anderson's observations are as follows:

A guinea-pig sensitized with milk, egg-white, and horse serum, either together or separately, will react with each of the three fluids given in the series. The blood of such a triply sensitized animal when injected into other animals will likewise render them passively sensitive to all three.<sup>13</sup>

It must be evident from this work that no method compares with anaphylaxis as a means of sharply distinguishing closely allied proteins. For the purpose of the experiments which form the basis of this paper, it is important to note that the digestion of protein seriously interferes with its giving the anaphylactic reaction. The most conclusive evidence on this point is afforded by the experiments of Wells,<sup>14</sup> who found that traces of coagulable protein remained after sixteen months' digestion with trypsin and that such almost completely digested protein would still sensitize to the homologous antigen, but only when very large doses were given, and even then the sensitization was so mild as not to give a fatal result. He states that

Pepsin-HCl digestion of egg albumin destroys its sensitizing and intoxicating properties very slowly, the former existing to a slight degree even after coagulable protein cannot be longer demonstrated. Conversion of egg albumin into acid albumin somewhat impairs, but by no means destroys, its powers to sensitize guinea pigs to egg albumin and to intoxicate pigs that have been sensitized with egg albumin.

He found, also, that as determined by the anaphylactic reaction, serum is much more resistant to tryptic digestion than egg albumin.

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12. Wells: Jour. Infect. Dis., 1911, ix, 147.

13. Rosenau and Anderson: Jour. Infect. Dis., 1908, iv, 552.

14. Wells: Jour. Infect. Dis., 1909, vi, 506.

The formation of acid albumin is, however, probably not accompanied by any considerable change of the molecule, and although such a process may be considered to be the first step to protein digestion, it is not to be compared with the cleavage action of the enzymes which break up the protein molecule into the various proteoses and amino-acids. These latter substances are to be considered as the true products of digestion in the gastro-intestinal tract, and Wells' experiments demonstrate that these substances

have no power to sensitize or intoxicate guinea-pigs, whether used in conjunction with themselves or with undigested egg-white. To quote further:

These experiments indicate that proteins cannot be decomposed much, if any, beyond the coagulable form without losing their anaphylactic properties. For the anaphylaxis reaction we must have intact protein molecules in soluble form. Possibly there exists a stage intermediate between the entire protein molecule and the ordinary proto- and duetero-proteoses, with which anaphylaxis can be produced, for coagulated proteins digested with either pepsin or trypsin show at certain stages a slight power to sensitize animals to egg albumin, but the power is very slight.

In the course of the experiments which are detailed below, the absorption of a variety of proteins has been tested—egg-white, milk, beef serum, horse serum and edestin. They have been introduced into the intestinal tract in some instances by means of the stomach-tube, or by hypodermic injection through the intestinal wall; in other cases into the stomach which had been ligated at both cardiac and pyloric ends or into ligated loops of the intestine, and in still others into Thiry-Vella fistulae, which were made in different portions of the intestine. It will be seen that both animal and vegetable proteins have been tested in normal conditions of absorption and in conditions in which some degree of impairment of digestive function may be presumed to exist.

A preliminary paper<sup>15</sup> has already been published giving the details of some of the earlier experiments, and from this paper such quotations will be made as have important bearing to the later work. The first point determined in the earlier paper was the question as to whether a foreign protein, egg albumin, could be injected directly into the circulation of a dog and remain there for several hours in such form that its presence could be detected by means of the anaphylactic reaction. The details of one of these experiments is quoted as follows:

A healthy dog was placed in a cage for a few days for observation and to obtain samples of urine. The animal was found to be well in all respects and the urine free from albumin. Under morphin and cocain anesthesia the jugular vein was aspirated and 100 c.c. of blood drawn to obtain normal serum. Following this procedure 20 c.c. of an egg albumin solution prepared as follows was injected into the vein: The whites of fresh eggs were thoroughly broken up and diluted with an equal volume of salt solution. The solution filtered through paper was ready for use. Fifteen minutes after the albumin was injected 20 c.c.

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15. Van Alstyne and Grant: Jour. Med. Research, 1911, xxv, 399.

of blood was withdrawn, and again at four hours, forty-eight hours, and seventy-two hours after the injection a further quantity was withdrawn. The serum from this blood was allowed to separate and kept for sensitizing purposes. The urine was collected daily and examined for albumin, and a portion of it kept for sensitizing purposes.

Guinea-pigs were sensitized with the serum and urine obtained as outlined above, the sensitizing dose varying from one-tenth cubic centimeter for the first twenty-four hours to five-tenths cubic centimeters for the seventy-two-hour period. One cubic centimeter of the urine was used for the same purpose. The sensitizing dose of serum was given subcutaneously and, after an appropriate period, in no case less than ten days, an intoxicating dose of the egg albumin solution, 2.5 or 3 c.c., was given intraperitoneally.

TABLE 1.—ANAPHYLACTIC REACTION IN GUINEA-PIGS PRODUCED BY BLOOD AND URINARY PROTEINS FROM DOGS WITH THIRY-VELLA FISTULAE

No. of Dog	No. of Guinea-Pig	Fistula	Substance Used for Sensitizing Dose	Toxic Dose	Results
675	273	Middle of small intestine	Serum drawn before albumin in fistula	Egg albumin	No symptoms
675	300	Middle of small intestine.	Serum drawn one hour thirty mins. after albumin in fistula	Egg albumin	Severe symptoms. Recovered
646	333	Eight inches below duodeno jejunal fossa.	Serum drawn three hours after albumin in fistula	Egg albumin	Severe symptoms. Recovered
667	612	Eight inches from cecum	Serum drawn one hour forty min. after introducing albumin in fistula	Egg albumin	Dead in forty-five minutes
...	354	Eight inches from cecum	*Albumin from urine	Egg albumin	Marked symptoms. Recovered
...	327	Eight inches below duodeno jejunal fossa	Urine from twelve to twenty-four hours after albumin in fistula	Egg albumin	Severe symptoms. Recovered

\* This pig was sensitized by giving him the protein obtained by one-half saturation of the urine with ammonium sulphate.

The results of this line of experimentation show that the serum of the dog to which the albumin was given intravenously was capable of sensitizing guinea-pigs to the subsequent dose of egg albumin. The egg albumin, therefore, was not immediately excreted or fixed in the tissues, but remained in the serum for at least three days in such concentration that five-tenths cubic centimeters of serum would sensitize a pig to a slight degree.

The egg albumin must have been excreted in the urine, for qualitative tests made of the first twenty-four-hour urine showed a small amount of albumin, and a pig sensitized with 1 c.c. of it gave a severe anaphylactic reaction.

All of the experiments in which dogs with a Thiry-Vella fistula were used have been published in a preliminary paper and the essential facts in these experiments will be quoted. The fistulae were made in three

different positions of the intestine: (1) Just below the duodeno-jejunal junction; (2) about the middle of the ileum, and (3) low down in the ileum. In the accompanying table (Table 1), which is condensed from the published preliminary paper, are given the experimental details and results obtained with those animals.

It does not seem possible to avoid the conclusion that the egg albumin had passed from the fistula into the circulation without alteration. These fistulae were made at a point in the intestine where the possibility of the formation of acid albumin is ruled out. It is not contended that these intestinal loops contained no digestive ferments or that a portion of the protein may not have been digested before absorption. The evidence is positive, however, that a portion of it entered the circulation without alteration and was excreted by the kidney in such form as to be precipitated by half saturation with ammonium sulphate. The amount of protein obtained from the urine was so small as to warrant the belief that a portion of the absorbed protein was utilized in the body. These experiments do not indicate how large a portion of the protein was absorbed without digestion, nor can it be assumed that absorption from a Thiry-Vella fistula is exactly identical with the absorption of protein from the same region of the intact intestine.

Since these experiments were positive, it seemed advisable to continue the investigation with somewhat different methods and with a variety of proteins. The question arose as to whether the same positive results would be obtained if the egg albumin should be introduced into the stomach by means of a tube. It can scarcely be assumed that such a method of introduction would in any measurable way interfere with the normal digestive processes. Accordingly, a dog was prepared in the usual fashion, blood was aspirated from the jugular vein before egg-white was introduced into the stomach, and preserved for further use. The whites of five eggs were beaten up with an equal quantity of salt solution and introduced into the stomach directly through a tube. Table 2 gives the experimental details.

The tabulated results indicate that either a small amount of egg albumin passed through the stomach walls without digestion or that some of it may have been absorbed as an acid albumin. Wells has shown that acid albumin sensitizes in slight degree to the unaltered protein. At any rate, it is fair to conclude that under these conditions only an exceedingly small portion of the ingested protein passed through unaltered.<sup>14</sup>

In the next experiment the technic was modified in that a ligature was placed around both the cardiac and pyloric ends of the stomach to insure the retention of the albumin in the organ. The experimental details are given in Table 3.



TABLE 2.—EXPERIMENTS IN ANAPHYLAXIS IN GUINEA-PIGS SENSITIZED WITH BLOOD AND URINARY PROTEINS FROM DOGS WITH THIRY-VELLA FISTULAE FED EGG ALBUMIN PER STOMACH

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incubation Days	Toxic Dose	Results
520	372	Eight min. dog serum before albumin introduced	21	(a) 3 c.c. egg albumin intraperitoneally. (b) 2 c.c. dog serum two days later	(a) No symptoms (b) Symptoms +
...	373	Eight min. dog serum one hour after albumin in stomach	21	(a) 2 c.c. egg albumin intraperitoneally. (b) 3 c.c. normal dog serum intraperitoneally two days.	(a) Mild symptoms (b) Symptoms +
...	374	Eight min. dog serum two hours after albumin in stomach	21	(a) 3 c.c. egg albumin intraperitoneally. (b) 3 c.c. normal dog serum intraperitoneally two days later	(a) Mild symptoms (b) Symptoms +
...	375	Eight min. dog serum three hours after albumin in stomach	21	(a) 3 c.c. egg albumin intraperitoneally. (b) 3 c.c. normal dog serum intraperitoneally two days later	(a) Mild symptoms (b) Dead two days later
...	376	Eight min. dog serum five hours after albumin in stomach	21	3 c.c. egg albumin intraperitoneally.	Mild symptoms
...	377	Eight min. dog serum twenty-four hours after alb. in stom.	21	3 c.c. egg albumin intraperitoneally.	No symptoms
...	558	Albumin from urine first twenty-four-hour period (dialysis)	21	3 c.c. egg albumin intraperitoneally.	No symptoms
...	516	Five c.c. dog serum drawn twenty-three days after albumin in stomach	21	3 c.c. egg albumin intraperitoneally.	No symptoms
...	518	Five c.c. dog serum drawn twenty-three days after albumin in stomach	21	3 c.c. egg albumin intraperitoneally.	No symptoms
...	515	One c.c. each one, two, three, five-hour bleedings plus 1 c.c. fresh normal dog serum.	21	1 c.c. egg albumin intravenously	Mild symptoms

Note 1.—In view of the fact that guinea-pigs often exhibit toxic symptoms which resemble some of the characteristic symptoms of anaphylaxis and that any guinea-pig may be restless and shake itself as the result of the handling incident to the intravenous injection of the toxic dose. I wish to state that in deciding in each instance whether the reaction is negative or mildly anaphylactic these facts were kept in mind and where there was any doubt, the result is stated as negative. Where the symptoms were typically anaphylactic, though mild, the results are stated as mild symptoms, and when more pronounced, such as the bucking movements, convulsions, marked drop in temperature, paralysis, etc., the results are stated as symptoms with one or two plus signs depending again on the severity of the symptoms.

Note 2.—I have appreciated from the beginning the necessity of taking every precaution to prevent contamination of the serum used for sensitizing purposes because of the exceedingly small amount necessary to sensitize an animal, and great care has been exercised to prevent such an occurrence. The syringes used to aspirate the blood were kept especially for this work and cleansed by me. The serum was kept in sterile, clean receptacles; the puncture wound made by the hypodermic needle in the stomach and intestines was closed by sutures.

The conditions are such in Experiment 3 that normal digestion cannot proceed and it will be readily seen that a much larger portion of the albumin passed into the circulation within a few hours after its introduction into the stomach. It is not claimed that this is an indication of the fate of albumin in normal digestion, but it indicates that an interference with the process which does not, during the time of experi-

TABLE 3.—EXPERIMENTS IN ANAPHYLAXIS. STOMACHS OF DOGS LIGATED TO INSURE RETENTION OF ALBUMIN

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incubation Days	Toxic Dose	Results
523	548	Eight min. dog serum before albumin in stomach	22	1 c.c. egg albumin injected into heart	No symptoms
...	582	Normal	22	1 c.c. egg albumin intravenously	No symptoms
...	549	Eight min. dog serum one hour after albumin in stomach	22	1 c.c. egg albumin intravenously	Mild symptoms
...	550	Eight min. dog serum two hours after albumin in stomach	22	1 c.c. egg albumin intravenously	Symptoms ++ Recovered
...	551	Eight min. dog serum three hours after albumin in stomach	22	1 c.c. egg albumin intravenously	Symptoms ++ Recovered
...	552	Eight min. dog serum five hours after albumin in stomach	22	2.0 c.c. egg albumin intravenously	Symptoms +++
...	554	One c.c. urine from bladder taken five hours after albumin in stomach	22	3 c.c. egg albumin intraperitoneally	Mild symptoms
..	518	One c.c. each serum one, two, three, five hours after albumin in stomach plus 1 c.c. fresh normal dog serum.	22	0.5 c.c. egg albumin intravenously	Symptoms +

Note.—Dog killed after last bleeding; bladder and stomach distended. Blood at five hours' bleeding dark in color and coagulated more quickly than one hour bleeding, although salt solution injected in sufficient quantities to cover fluid loss from bleeding.

ment, seriously injure the vitality of the mucosa, permits the protein to pass through.

In the next experiment a portion of the colon just below the ileocecal valve was ligated and egg albumin injected into the ligated portion.

The results again show (Table 4) a very marked absorption of the albumin without digestion. The conditions are not very different in this experiment from those which might be obtained in a case of intestinal

obstruction. The blood- and nerve-supply to this loop was not seriously disturbed, except in those tissues immediately under the ligature.

In the next experiment the question arose as to whether the ligation of the colon is an important factor in the rapid absorption of the undigested protein. Accordingly, Experiment 4 was repeated, except that

TABLE 4.—RESULTS OF EXPERIMENT SIMILAR TO PREVIOUS ONES. EGG ALBUMIN INJECTED INTO LIGATED PORTION OF COLON

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incubation Days	Toxic Dose	Results
526	810	One c.c. dog serum before albumin injected	27	1 c.c. egg albumin inj. intravenously	No symptoms
...	977	One c.c. dog serum before albumin injected	27	1 c.c. egg albumin inj. intravenously	No symptoms
...	978	One c.c. dog serum before albumin injected	27	1 c.c. egg albumin inj. intravenously	No symptoms
...	811	One c.c. dog serum one hour after albumin injected	27	1 c.c. egg albumin inj. intravenously	Symptoms + + +. Recovered and died five days later
...	813	One c.c. dog serum two hours after albumin injected	27	1 c.c. egg albumin inj. intravenously	Symptoms + +
...	814	One c.c. dog serum three hours after albumin injected	27	1 c.c. egg albumin inj. intravenously	Symptoms + +
...	815	One c.c. dog serum four hours after albumin injected	27	1 c.c. egg albumin inj. intravenously	Symptoms + +
...	816	One c.c. dog serum five hours after albumin injected	27	1 c.c. egg albumin inj. intravenously	Dead in three minutes
...	821	One c.c. urine drawn from bladder six hours after albumin injection	27	1 c.c. egg albumin inj. intravenously	Symptoms +
...	979	One and one-half c.c. dog serum 3 hours after alb. injected.	27	1 c.c. egg albumin inj. intravenously	Symptoms + +
...	980	One c.c. dog serum four hours after albumin injected	27	1 c.c. egg albumin inj. intravenously	Symptoms + +

the egg albumin was injected into the colon below the ileo-cecal valve, but no ligatures were placed about the colon. The abdominal wall was carefully sutured and blood drawn at the intervals indicated. In Table 5 is shown the experimental details.

From these results it is justifiable to conclude that absorption of undigested protein is facilitated by ligation of the portion of the intestine

TABLE 5.—RESULTS IN EXPERIMENT FIVE. TECHNIC SIMILAR TO THAT IN EXPERIMENT FOUR, EXCEPT THAT COLON WAS NOT LIGATED

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incubation Days	Toxic Dose	Results
400	449	Two c.c. dog serum before egg albumin injected	23	1 c.c. egg white intravenously	No symptoms
...	463	Two c.c. dog serum before egg albumin injected	23	1 c.c. egg white intravenously	No symptoms
...	475	Two c.c. dog serum one hour after egg albumin injected	23	1 c.c. egg white intravenously	Slight symptoms
...	492	Two c.c. dog serum one hour after egg albumin injected	23	1 c.c. egg white intravenously	Symptoms
...	478	Two c.c. dog serum one hour after egg albumin injected	23	1 c.c. egg white intravenously	Slight symptoms. Dead eight hours
...	420	Two c.c. dog serum two hours after egg albumin injected	23	1 c.c. egg white intravenously	Slight symptoms
...	441	Two c.c. dog serum two hours after egg albumin injected	23	1 c.c. egg white intravenously	No symptoms
...	410	Two c.c. dog serum two hours after egg albumin injected	23	1 c.c. egg white intravenously	No symptoms
...	431	Two c.c. dog serum three hours after egg albumin injected	23	1 c.c. egg white intravenously	No symptoms
...	459	Two c.c. dog serum three hours after egg albumin injected	23	1 c.c. egg white intravenously	No symptoms
...	457	Two c.c. dog serum three hours after egg albumin injected	23	1 c.c. egg white intravenously	No symptoms
...	484	Two c.c. dog serum four hours after egg albumin injected	23	1 c.c. egg white intravenously	No symptoms
...	447	Two c.c. dog serum four hours after egg albumin injected	23	1 c.c. egg white intravenously	No symptoms



into which the protein is injected. These results agree with those obtained in the stomach experiments and demonstrate that obstruction to the normal peristaltic action is an important factor in permitting the passage of foreign albumin through the mucosa. Attention is drawn to the fact that as regards absorption, the ligated loop behaves very much like a Thiry-Vella fistula in a similar portion of the intestine. When a portion of the intestine is denied free access to the secretions formed in other portions of the intestine there is apparently more absorption of the unaltered protein. Since the Thiry-Vella fistula resembles a ligated portion of the intestine in its absorption phenomena, it seems probable

TABLE 6.—ANAPHYLACTIC EXPERIMENTS WITH MILK

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incubation Days	Toxic Dose	Results
557	559	Eight min. dog serum before milk in stomach	29	1 c.c. milk injected intravenously	No symptoms
...	560	Eight min. dog serum one hour after milk in stomach.	29	1 c.c. milk injected intravenously	Mild symptoms
...	561	Eight min. dog serum two hours after milk in stomach.	29	1 c.c. milk injected intravenously	* Dead in four hours
...	564	Eight min. dog serum three hours after milk in stomach	29	1 c.c. milk injected intravenously	Mild symptoms
...	563	Eight min. dog serum five hours after milk in stomach	29	1 c.c. milk injected intravenously	Mild symptoms

\* Death probably due to the fact that ether was used during operation on the animal. In no other instance was any anesthetic used.

that the lack of digestive enzymes from other portions of the intestine is quite as potent a factor as the obstruction.

The success of these experiments led me to try the absorption with another form of protein which is one of the most important food-stuffs, namely, milk. Milk is readily digested. The protein of the milk is not as toxic in anaphylactic experiments as that found in the egg or serum proteins, but many experimenters have used it in anaphylactic experiments. The details of the first experiment are given in Table 6.

The milk, in this instance, was put in the stomach through a tube. Physiological conditions prevailed and yet the serum of the dog from one to five hours after the milk was put into the stomach was capable of sensitizing guinea-pigs.

In the next experiment the abdomen was opened, the cardiac and pyloric ends ligated and the milk introduced into the stomach directly through a large hypodermic needle. The experimental details are given in Table 7.

TABLE 7.—ANAPHYLACTIC EXPERIMENT WITH MILK. STOMACH LIGATED AT BOTH ENDS AND MILK INTRODUCED DIRECTLY THROUGH A NEEDLE

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incubation Days	Toxic Dose	Results
565	567	Eight min. dog serum before milk in stomach	29	1 c.c. milk injected intravenously	No symptoms
...	568	Eight min. dog serum one hour after milk in stomach	29	1 c.c. milk injected intravenously	Symptoms +
...	570	Eight min. dog serum three hours after milk in stomach	29	1 c.c. milk injected intravenously	Symptoms +
...	582	Eight min. dog serum five hours after milk in stomach	29	1 c.c. milk injected intravenously	Symptoms +
...	573	Eight min. dog serum twenty-four hours after milk in stomach	29	1 c.c. milk injected intravenously	Symptoms +
...	575	One c.c. urine drawn before milk in stomach	29	1 c.c. milk injected intravenously	No symptoms
...	706	Two c.c. urine twenty-four hours after milk in stom.	29	1 c.c. milk injected intravenously	Symptoms +
...	583	Two c.c. urine forty-eight hours after milk in stomach	29	1 c.c. milk injected intravenously	Mild symptoms
...	582	Eight min. dog serum five hours after milk in stomach	29	1 c.c. milk injected intravenously	Symptoms +
...	584	Eight min. dog serum forty-eight hours after milk in stomach	29	1 c.c. milk injected intravenously	Mild symptoms

Note—Dog killed after forty-eight hours' bleeding: stomach greatly distended.

In this case it appears that more of the milk than in the previous experiment must have been absorbed directly into the circulation, for the guinea-pigs were more actively sensitized than before. This corresponds to the finding in the similar experiment with the egg albumin. Evidently when the stomach is prevented from emptying itself, undigested protein is absorbed more readily.

In the next experiment the duodenum was ligated, injected with milk, with the somewhat surprising result shown in Table 8.

The serum taken from two to five hours after milk was introduced did not sensitize guinea-pigs. It seems probable that in the duodenum the digestive action has been sufficient to split the protein to such an extent that it no longer sensitized to the homologous protein.

Since this experiment seems to contradict the results of the experiments with egg albumin reported above, attention may be called to the fact that the portion of the duodenum ligated is well supplied with digestive enzymes; a condition quite contrary to that obtained in lower portions of the intestine.

TABLE 8.—DUODENUM LIGATED AND MILK INJECTED DIRECT

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incubation Days	Toxic Dose	Results
518	974	Two c.c. dog serum before	21	5 c.c. milk injected intravenously	No symptoms
...	975	Two c.c. dog serum two hours after milk injected	21	(a) 5 c.c. milk injected intravenously (b) 1 c.c. normal dog serum three days later	(a) No symptoms (b) Symptoms ++
...	976	Two c.c. dog serum two hours after milk injected	21	(a) 5 c.c. milk injected intravenously (b) 1 c.c. normal dog serum three days after	(a) No symptoms (b) Symptoms ++
...	820	Eight min. dog serum five hours after milk injected	21	5 c.c. milk injected intravenously	No symptoms
...	972	Three c.c. urine three hours after milk injected	21	5 c.c. milk injected intravenously	No symptoms

NOTE.—This dog died twelve hours after operation; ligated duodenum greatly distended and ulcerated.

In the next three experiments, Tables 9, 10 and 11, horse serum was used as the antigen.

In Experiment 9 the horse serum was introduced into the stomach through the stomach tube; in Experiment 10 the serum was introduced into the unligated portion of the upper small intestine; in Experiment 11 the antigen was injected into a ligated loop made in about the middle of the small intestine. In Tables 9, 10 and 11 are given the experimental details and results.

From these results it will be seen that there was a slight amount of absorption from the unligated stomach, no absorption from the unligated

TABLE 9.—RESULTS OF EXPERIMENTS IN WHICH HORSE SERUM WAS USED AS THE ANTIGEN INTRODUCED DIRECTLY INTO THE STOMACH

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incubation Days	Toxic Dose	Results
402	301	Two c.c. dog serum two hours after horse serum introduced	22	1 c.c. inactivated horse serum intravenously	Slight symptoms
...	323	Two c.c. dog serum two hours after horse serum introduced	22	1 c.c. inactivated horse serum intravenously	Symptoms
...	309	Two c.c. dog serum two hours after horse serum introduced	22	1 c.c. inactivated horse serum intravenously	Slight symptoms
...	319	Two c.c. dog serum two hours after horse serum introduced	22	1 c.c. inactivated horse serum intravenously	Slight symptoms
...	303	Two c.c. dog serum five hours after horse serum introduced	22	1 c.c. inactivated horse serum intravenously	Slight symptoms
...	305	Two c.c. dog serum five hours after horse serum introduced	22	1 c.c. inactivated horse serum intravenously	Slight symptoms
...	307	Two c.c. dog serum five hours after horse serum introduced	22	1 c.c. inactivated horse serum intravenously	Slight symptoms
...	321	Two c.c. dog serum five hours after horse serum introduced	22	1 c.c. inactivated horse serum intravenously	Slight symptoms
...	317	Two c.c. dog serum twenty-two hours after horse serum introduced.	22	1 c.c. inactivated horse serum intravenously	No symptoms
...	315	Two c.c. dog serum twenty-two hours after horse serum introduced	22	1 c.c. inactivated horse serum intravenously	Slight symptoms
...	311	Two c.c. dog serum twenty-two hours after horse serum introduced	22	1 c.c. inactivated horse serum intravenously	No symptoms
...	313	Two c.c. dog serum twenty-two hours horse serum introduced	22	1 c.c. inactivated horse serum intravenously	No symptoms



TABLE 10.—RESULTS OF EXPERIMENT WHEN HORSE SERUM WAS INTRODUCED INTO UNLIGATED PORTION OF UPPER SMALL INTESTINE

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incubation Days	Toxic Dose	Results
200	363	Two c.c. dog serum one and one-half hours after horse serum injected	23	1 c.c. inactivated horse serum intravenously	No symptoms
...	339	Two c.c. dog serum one and one-half hours after horse serum injected	23	1 c.c. inactivated horse serum intravenously	No symptoms
...	345	Two c.c. dog serum two hours after horse serum injected	23	1 c.c. inactivated horse serum intravenously	No symptoms
...	347	Two c.c. dog serum four hours after horse serum injected	23	1 c.c. inactivated horse serum intravenously	No symptoms
...	341	Two c.c. dog serum four hours after horse serum injected	23	1 c.c. inactivated horse serum intravenously	No symptoms
...	359	Two c.c. dog serum four hours after horse serum injected	23	1 c.c. inactivated horse serum intravenously	No symptoms

Note.—Remainder of series not injected, as one and one-half, two- and four-hour animals were all negative.

TABLE 11.—RESULTS WHEN ANTIGEN WAS INJECTED INTO A LIGATED LOOP OF SMALL INTESTINE

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incubation Days	Toxic Dose	Results
392	783	One c.c. dog serum before horse serum injected	29	1 c.c. horse serum intravenously	No symptoms
...	788	One c.c. dog serum before horse serum injected	29	1 c.c. horse serum intravenously	No symptoms
...	784	One c.c. dog serum one hour after horse serum injected	29	1 c.c. horse serum intravenously	Symptoms +
...	785	One c.c. dog serum one hour after horse serum injected	29	1 c.c. horse serum intravenously	Symptoms +
...	790	One c.c. dog serum two hours after horse serum injected	29	1 c.c. horse serum intravenously	Symptoms ++

upper intestine and marked absorption from the ligated loop. In practically all respects these three experiments show that horse serum behaves like egg albumin in its absorption capacities.

In the next two experiments beef serum was used as an antigen. In both instances the serum was injected into the ligated lower portion of the ileum. Since the results of the two experiments were identical, only one will be quoted. The results are seen in Table 12.

The results of this experiment are identical with those in which other proteins were used as antigens.

TABLE 12.—RESULTS OF EXPERIMENT IN WHICH BEEF SERUM ANTIGEN WAS INJECTED INTO LIGATED LOWER PORTION OF ILEUM

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incubation Days	Toxic Dose	Results
833	897	Two c.c. dog serum before beef serum injected	25	1.5 c.c. beef serum intravenously	No symptoms
...	898	Three c.c. dog serum before beef serum injected	25	1 c.c. beef serum intravenously	No symptoms
...	899	One c.c. dog serum before beef serum injected	25	1 c.c. beef serum intravenously	No symptoms
...	211	Normal guinea pig	25	1 c.c. beef serum intravenously	No symptoms
...	900	One c.c. dog serum one hour after beef serum injected	25	1 c.c. beef serum intravenously	Symptoms +
...	894	Two c.c. dog serum one hour after beef serum injected	25	1 c.c. beef serum intravenously	Symptoms +
...	895	One c.c. dog serum two hours after beef serum injected	25	1 c.c. beef serum intravenously	Dead in five minutes
...	892	Two c.c. dog serum two hours after beef serum injected	25	1 c.c. beef serum intravenously	Dead in fifteen minutes

All of the proteins used in the experiments thus far reported have been of animal origin, and it is quite possible that vegetable proteins may not have the same absorption behavior as those of animal origin. In the two following experiments, edestin<sup>16</sup> was used as the antigen. Two experiments were made, in both of which the solution of edestin was injected into the ligated lower portion of the ileum. Since the two experiments agree as to results, only one of them will be quoted. In Table 13 is given the details of one of these experiments.

16. The edestin used in these experiments was prepared by Dr. Martha Tracy in Professor Mendel's laboratory.

From the results recorded above, one point is evident: the absorption of the edestin is accomplished more slowly than in the case of the animal antigens. The symptoms given by pigs sensitized with the blood drawn during the earlier hours following the injection are mild; the severer symptoms are given by pigs sensitized with blood drawn four hours after the introduction of the antigen. The reason for this is probably the slow

TABLE 13.—RESULTS OF EXPERIMENT IN WHICH EDESTIN IN SOLUTION WAS INJECTED INTO LIGATED LOWER PORTION OF ILEUM

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incubation Days	Toxic Dose	Results
434	438	One c.c. dog serum before edestin injected	23	1 c.c. edestin injected intravenously	No symptoms
...	440	One c.c. dog serum before edestin injected	23	1 c.c. edestin injected intravenously	No symptoms
...	442	One c.c. dog serum before edestin injected	23	1 c.c. edestin injected intravenously	No symptoms
...	444	Two c.c. dog serum one hour after edestin injected	23	1 c.c. edestin injected intravenously	Mild symptoms
...	446	Two c.c. dog serum two hours after edestin injected	23	1 c.c. edestin injected intravenously	Mild symptoms
...	448	Two c.c. dog serum two hours after edestin injected	23	1 c.c. edestin injected intravenously	Mild symptoms
...	450	Two c.c. dog serum three hours after edestin injected	23	1 c.c. edestin injected intravenously	Mild symptoms
...	452	Two c.c. dog serum three hours after edestin injected	23	1 c.c. edestin injected intravenously	Mild symptoms
...	454	Two c.c. dog serum three hours after edestin injected	23	1 c.c. edestin injected intravenously	Mild symptoms
...	456	Two c.c. dog serum four hours after edestin injected	23	1 c.c. edestin injected intravenously	Mild symptoms
...	458	Two c.c. dog serum four hours after edestin injected	23	1 c.c. edestin injected intravenously	Symptoms +
..	460	Two c.c. dog serum four hours after edestin injected	23	1 c.c. edestin injected intravenously	Symptoms +

Note 1.—Total mg. edestin injected into loop —2.750 mg. protein.

Note 2.—Dog killed and autopsied after last (four hours) bleeding. Ligated portion empty except for small quantities of fecal matter.

Note 3.—Sixty c.c. blood drawn; 90 c.c. NaCl injected in two portions at second and third bleedings. Blood at third and fourth bleedings dark in color and clotted very quickly.

absorption of the antigen, which is probably due to the fact that soon after the solution was introduced into the intestine it was precipitated. Other investigators have noted that edestin is readily precipitated from its solution.<sup>10</sup>

In the following experiment a point which is somewhat incidental to the main question has been subjected to experimental investigation. In the first experiments which were made on this topic, a preliminary report of which has already been published, I found that egg albumin could be detected in the blood for three days after the time of injection, but not beyond this time. It seemed advisable to further test this observation with two types of protein, edestin and horse serum. In the first experiment edestin was used as the antigen. Fifty cubic centimeters of a solution containing altogether 3.1 gm. of protein was injected into the jugular vein of a dog. The animal was bled three, six, ten, thirteen and sixteen days after the injection was made. Further experimental details are given in Table 14.

From this table it may be seen that the only pigs that were sensitized to the edestin were those receiving blood withdrawn three days after the injections were made. It follows that the edestin must have remained in the dog's circulation for a period of at least three days, but not as long as six days.

Both egg-white and edestin are proteins so different in structure from blood serum that it is quite possible that they might be removed from the circulation in a shorter time than a serum protein from another species of animal. After an injection of diphtheria antitoxin antibodies have been found in the blood as late as three weeks after the injection. The length of time in which horse serum would remain in the circulation of the dog was tested on three different dogs. Fifty cubic centimeters of horse serum were injected into the jugular vein. Blood was drawn at different periods up to forty-nine days and used to sensitize guinea-pigs. Inasmuch as the dog may have become immune to the antigen, it was necessary in this case to test for passive anaphylaxis. The experimental details in one case are given in Table 15.

Attention is called to the fact that six pigs sensitized with the serum drawn forty-nine days after horse serum was injected into the dog were tested for passive anaphylaxis, but only one showed any symptoms. These were very mild, while four other pigs that were allowed to incubate for a period of forty-one days, when tested for active anaphylaxis showed marked reactions, one of them dying in ten minutes.

It seems probable that the horse serum has remained in the dog's circulation unaltered during this time.

The behavior of the horse serum is distinctly different from that of the edestin or the egg albumin. It would seem that these latter proteins



are much more promptly attacked and removed from the circulation than is serum protein.

This experiment is somewhat similar to that of Gay and Southard,<sup>17</sup> who bled guinea-pigs that had been previously used in diphtheria anti-

TABLE 14.—EXPERIMENT WITH EDESTIN AS ANTIGEN TO DETERMINE HOW LONG THE FOREIGN PROTEIN REMAINED IN THE BLOOD

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incubation Days	Toxic Dose	Results
505	423	Two c.c. dog serum ten days after edestin injected	6	1.5 c.c. solution edestin inj. intravenously	No symptoms
...	401	One c.c. dog serum before edestin injected	46	1 c.c. edestin injected intravenously	No symptoms
...	403	One c.c. dog serum before edestin injected	46	1 c.c. edestin injected intravenously	No symptoms
...	405	One c.c. dog serum before edestin injected	46	1 c.c. edestin injected intravenously	No symptoms
...	407	Two c.c. dog serum three days after edestin injected	46	1 c.c. edestin injected intravenously	Mild symptoms
...	409	Two c.c. dog serum three days after edestin injected	46	1 c.c. edestin injected intravenously	Mild symptoms
...	411	Two c.c. dog serum three days after edestin injected	46	1 c.c. edestin injected intravenously	Mild symptoms
...	413	Two c.c. dog serum six days after edestin injected	46	1 c.c. edestin injected intravenously	No symptoms
...	415	Two c.c. dog serum six days after edestin injected	46	1 c.c. edestin injected intravenously	No symptoms
...	417	Two c.c. dog serum six days after edestin injected	46	1 c.c. edestin injected intravenously	No symptoms
...	423	Two c.c. dog serum ten days after edestin injected	46	1 c.c. edestin injected intravenously	No symptoms
...	167	Two c.c. dog serum thirteen days after edestin injected	46	1 c.c. edestin injected intravenously	No symptoms
...	210	Two c.c. dog serum thirteen days after edestin injected	46	1 c.c. edestin injected intravenously	No symptoms

toxin experiments, 204 and 169 days after their injection of horse serum, and injected this serum into other normal guinea-pigs. Fifteen days later the pigs were injected with horse serum with fatal results. Since

17. Gay and Southard: Jour. Med. Research, 1907, xvi, 143.

TABLE 15.—EXPERIMENT WITH HORSE SERUM AS ANTIGEN TO DETERMINE HOW LONG THE FOREIGN PROTEIN REMAINED IN THE BLOOD

No. of Dog	No. of Guinea-Pig	Sensitizing Dose	Period of Incu- bation Days	Toxic Dose	Results
320	109	Two c.c. dog serum thirty-one days after horse serum injected	1	1 c.c. horse serum intravenously	No symptoms
...	945	Two c.c. dog serum thirty-one days after horse serum injected	2	1 c.c. horse serum intravenously	No symptoms
...	308	Two c.c. dog serum thirty-one days after horse serum injected	2	1 c.c. horse serum intravenously	No symptoms
...	502	Two c.c. dog serum thirty-one days after horse serum injected	3	1 c.c. horse serum intravenously	No symptoms
...	264	Two c.c. dog serum before horse serum injected	25	1 c.c. horse serum intravenously	No symptoms
...	952	Two c.c. dog serum before horse serum injected	25	1 c.c. horse serum intravenously	No symptoms
...	880	Two c.c. dog serum before horse serum injected	25	1 c.c. horse serum intravenously	No symptoms
...	571	Two c.c. dog serum thirty-one days after horse serum injected	25	1 c.c. horse serum intravenously	Symptoms +
...	927	Two c.c. dog serum thirty-one days after horse serum injected	25	1 c.c. horse serum intravenously	Symptoms +
...	946	Two c.c. dog serum thirty-one days after horse serum injected	25	1 c.c. horse serum intravenously	Symptoms ++
...	847	Two c.c. dog serum forty-nine days after horse serum injected	4	1 c.c. horse serum intravenously	No symptoms
...	212	Two c.c. dog serum forty-nine days after horse serum injected	4	1 c.c. horse serum intravenously	Symptoms (?)
...	162	Two c.c. dog serum forty-nine days after horse serum injected	41	1 c.c. horse serum intravenously	Symptoms +
...	839	Two c.c. dog serum forty-nine days after horse serum injected	41	1 c.c. horse serum intravenously	Symptoms +
...	968	Two c.c. dog serum forty-nine days after horse serum injected	41	1 c.c. horse serum intravenously	Symptoms ++
...	705	Two c.c. dog serum forty-nine days after horse serum injected	41	1 c.c. horse serum intravenously	Died in ten minutes

Note—As the serum of the six-, ten- and thirteen-day pigs was negative, the sixteen-day pigs were not intoxicated.

the authors did not test these pigs for passive sensitization, and since it is known that passive sensitization with the serum of an homologous animal lasts for a long time, there is no reason to suppose that the experimental pigs were not passively rather than actively sensitized. In our case, however, it is known that the active sensitization was much stronger than the passive sensitization.

#### SUMMARY

1. These experiments demonstrate that protein may be absorbed unaltered through the intact epithelium of the gastro-intestinal tract.

2. Conditions which interfere with normal digestive function, such as ligaturing a portion of the intestine, or the whole stomach, or the isolation of a portion of the intestine as a Thiry-Vella fistula, markedly increase the amount of protein absorbed.

3. The amount of protein absorbed without digestion under physiological conditions is too small to be of any significance as a factor in nutrition.

4. Edestin and egg albumin when injected intravenously into a dog do not remain in the circulation in a condition to sensitize guinea-pigs for a long period; in our experiments for three days. On the other hand, horse serum may be detected in the dog's circulation for a period of at least forty-nine days.

5. The absorption in the duodenum differs from that in other portions of the intestine in that the ligation of the duodenum does not facilitate absorption of unaltered protein.

Although the amount of unaltered protein concerned in this reaction is not large, it may, nevertheless, be of decided import. Only a very small amount of protein is required to sensitize an animal, or seriously intoxicate a sensitive animal. Certain pathological conditions may have their origin in the fact that the absorbed protein has caused certain toxic reactions. The relation between anaphylaxis and asthma has been pointed out many times. It is well known that asthmatic persons are often particularly sensitive to certain food-stuffs. Many skin conditions are classed by dermatologists as anaphylactic in their nature.<sup>18</sup> There is considerable possibility that these experiments offer an explanation for such manifestations. Certain food intoxications, and some of the symptoms of intestinal obstruction, may be in part dependent on the absorption of unaltered protein.

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18. Johnson: *Jour. Cutan. Dis.*, 1912, xxx, 136.

# THE CHOLESTEROL AND CHOLESTEROL-ESTER CONTENT OF THE BLOOD IN XANTHOMA TUBEROSUM MULTIPLEX \*

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## I. INTRODUCTION

In recent years considerable interest has been shown regarding the rôle that cholesterol and its esters play in the body. Cholesterol was first noted by Conradi in 1775 and by Gren in 1788, to be present in gall-stones, and they called this substance "gall-stone fat." Fourcroy subsequently classified it along with spermaceti and adipocere. Chevreul, in 1815, demonstrated certain differential qualities of this substance and called it cholesterin.

This substance, now preferably called cholesterol ( $C_{27}H_{44}O \cdot H_2O$ ), is a mono-hydroxy terpeno-alcohol and forms esters with fatty acids in the same manner as does the tri-hydroxy alcohol glycerol. The esters of cholesterol with oleic and palmitic acids exist preformed in animal tissues, while that with stearic acid has not been identified positively in organisms. Bondet, in 1883, found cholesterol-oleate in the blood-serum and called it "seroline," but Hürthle, in 1895, was the first to identify and study the substance. This ester has also been found normally and pathologically in many portions of the body, in the kidneys, liver, arteriosclerotic arteries, etc., but is absent from the brain.

Some of the esters of cholesterol show a crystalline fluid phase (fluid crystals) and the identification of such substances by Kaiserling and Orgler<sup>1</sup> first directed modern attention to this peculiar phenomenon. They have also been carefully studied by Aschoff,<sup>2</sup> Adami,<sup>3</sup> Kawamura<sup>4</sup> and others.

In view of the fact that several observers have considered an excess of cholesterol esters in the blood to be present in the condition called xanthoma tuberosum multiplex, I thought it would be of considerable

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\* From the Biochemical Laboratory of the Western Pennsylvania Hospital, Pittsburgh, Pa.

\* Accepted for publication June 14, 1913.

1. Kaiserling and Orgler: *Virchows Arch. f. path. Anat.*, 1902, clxvii; *Berl. klin. Wehnschr.*, 1910, xlvii, 2156; *Deutsch. med. Wehnschr.*, 1910, 1466.

2. Aschoff: *Beitr. z. path. Anat.*, 1910, xlvii, 1.

3. Adami: *The Myelins and Potential Fluid Crystalline Bodies of the Organisms*, The Harvey Lectures, 1908, No. 2. p. 117.

4. Kawamura: *Die Cholesterinester-Verfettung*, Jena., 1911.



interest to attack this problem from an experimental standpoint, especially as the new method of Windaus for the estimation of the cholesterol and cholesterol-esters is very accurate, and also because in my case the samples of blood were collected for analysis during the time when new xanthoma lesions were forming, giving every reason to suppose that if the condition was characterized by an excess of cholesterol esters in the blood, this would be the most favorable time to find the same.<sup>5</sup>

I would also draw attention to the fact that in my work, a "control" was studied. This person was convalescent from an attack of gonorrheal arthritis, and for two days previous to the collection of the blood was taking the same diet as the patient with xanthoma tuberosa multiplex.

## II. REVIEW OF LITERATURE

Pinkus and Pick<sup>6</sup> made studies of the common flat xanthoma of the eyelids; xanthoma tuberosum diabeticum, xanthoma tuberosum in diabetes and xanthoma tumor, a large isolated tumor. Pathologically, they found each of these types to be composed of the same cellular elements and that microchemically and microphysically they were identical. Examination of unstained frozen sections hardened in formaldehyd solution showed the entire tissue to be filled with fine needle-like crystals which showed the phenomena of "anisotropism" when examined under a polarizing microscope. With osmic acid there was almost no blackening. Sudan III stained the tissue red or brown-red.<sup>7</sup>

They concluded that the characteristic substance of xanthomas is not fat, but the doubly refracting substance. They think it resembles the so-called "protagon" and is a cholesterol-ester, which substance is supposed to be found in the blood in icterus and diabetes in greatly increased amounts and that the deposition of this substance in the tissues produces the formation of xanthoma.

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5. I wish to thank Dr. H. Fox, dermatologist to the German Hospital, New York, for his kindness in placing the patient at my disposal. The samples of blood were collected and the drying of the same was done at the German Hospital. The rest of the work was carried out in Pittsburgh.

6. Pinkus and Pick: *Deutsch. med. Wehnschr.*, 1908, xxxiv, 1426; *Monatsch. f. prakt. Dermat.*, 1908, xlv; *Arch. f. Derm.*, 1908, cxii, 228.

7. Through the kindness of Dr. S. Pollitzer I had the opportunity of studying certain properties of the coloring matter present in the xanthoma cell. The material used was obtained at biopsy from Dr. Pollitzer's patient. It was ground up with sand and when treated with ether, imparted a golden color to the ether. This solution gave all the color reactions typical of lipochromes, and readily diffused from ether through a rubber membrane into ether, a property which I have found lipochromes to possess. Exposure of an ethereal solution of this lipochrome to direct sunlight for a period of three hours caused complete disappearance of the color, and on adding various oxidizing agents, the color did not return. For the above reasons we can conclude that this coloring matter is a true lipochrome.

Pringsheim<sup>8</sup> examined xanthomatous tissues from the skin and dura mater chemically and found that certain acetone-soluble crystals present were free from nitrogen and phosphorus and gave all the reactions peculiar to cholesterol, and that they had a constant melting point. He believes this substance to be the same as that described by Pinkus and Pick and also thinks that the substance is deposited in xanthoma by cholesterol from the blood.

Chauffard and Laroche<sup>9</sup> claim that xanthoma is probably caused by an increase of greater or less duration in the cholesterol content of the blood-serum, and conclude, therefore, that it is a sign of lipolytic insufficiency of the pancreas and is less to be regarded as a tumor than as a local reaction of the skin to the cholesterol present in the blood-serum.

Pollitzer and Wile<sup>10</sup> have found in the earliest lesions of xanthoma, an infiltration of the perivascular and intercellular lymph-spaces with a fatty substance, which is present to a considerable extent also in the new-formed cells, and in the endothelium of the capillaries and here and there in the basal layer of the epidermis. The xanthoma giant cells on staining with osmic acid or Sudan III are found to be almost entirely filled with a fatty substance. This substance was also soluble in alcohol. On examination of fresh sections of xanthoma by polarized light, they found that at least a greater portion of the fatty substance contained in the cells presented the phenomena of anisotropism. They think it likely that in cases associated with an excess of lipins in the blood, that the lipins in excess pass out through the capillaries of the skin at some point of lessened resistance, and their presence in the perivascular connective tissue cells appears to act as a stimulus and causes a proliferation of these cells, which take up the lipins poured out from the blood-vessels and form the xanthoma cells.

They concluded that xanthoma tuberosum represents an irritative connective-tissue hyperplasia, in which the extravasation of cholesterol fatty acid-ester present in excess in the blood serves as the stimulus, and that certain connective tissue cells which take up these substances proliferate and increase in size, forming the typical xanthoma cells.

### III. COLLECTION AND ANALYSES OF THE BLOOD

The blood was obtained from the median basilic vein by means of a Wassermann needle. It was collected in a weighed bottle containing ammonium oxalate to prevent coagulation, the increase in weight of the bottle giving the amount of blood collected. The blood was then cen-

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8. Pringsheim: *Deutsch. med. Wehnschr.*, 1908, xxxiv, 2145; *Biochem. Ztschr.*, 1908, xv, 52.

9. Chauffard and Laroche: *Zentralbl. f. Biochem. u. Biophys.*, 1912, xiii, 102.

10. Pollitzer and Wile: *Jour. Cutan. Dis.*, 1912, xxx, 235.

trifuged, and the corpuscles washed thoroughly with physiological salt solution and the washings added to the plasma. The corpuscles and plasma were then dried separately by means of anhydrous sodium sulphate, placed in a Soxhlet extractor and extracted with anhydrous ether, followed by extraction with alcohol and then with ether, until all the ether and alcohol soluble substances were extracted.

The extracts were evaporated in a weighed dish at 35 C., and then placed in a vacuum desiccator with sulphuric acid until of constant weight. The cholesterol and cholesterol-esters present were then estimated by the excellent method of Windaus.<sup>11</sup>

TABLE SHOWING CHOLESTEROL AND CHOLESTEROL-ESTER CONTENT OF THE BLOOD PER 100 GRAMS OF BLOOD\*

Case	Blood Corpuscles		Blood Plasma	
	Cholesterol, Per Cent.	Cholesterol- Ester, Pct.	Cholesterol, Per Cent.	Cholesterol- Ester, Pct.
Xanthoma tuberosa multiplex† .....	0.065	<i>trace</i>	0.048	0.022
Control‡ .....	0.068	<i>trace</i>	0.046	0.028

\* Cholesterol-ester figures are in terms of the stearate.

† 26.8670 grams of blood used for the analyses.

‡ 25.4104 grams of blood used for the analyses.

The accompanying table contains the results obtained in this study, showing that in the case of xanthoma tuberosum multiplex studied, there was *no increase in the amount of cholesterol or of cholesterol-esters in the corpuscles or plasma when compared with the corpuscles and plasma of a normal person as a control.*

The writer wishes to thank Dr. F. M. Hanes for giving him the expensive digitonin that was used in this method of estimation of these substances.

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11. Windaus: Ber. d. deutsch. chem. Gesellsch., 1909, xlii, 238; Ztschr. f. physiol. Chem., 1910. lxx, 110.

## THE RELATION OF FATIGUE TO PARALYSIS LOCALIZATION IN PLUMBISM \*

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The apparent predilection of lead and its salts for the extensor muscles of the wrist, or the musculospiral nerve which supplies them, has for a long time remained unexplained.

This peculiar localization, as well as changes in the muscles themselves, has formed subject matter for numerous hypotheses. One of the oldest views, which is now almost obsolete, held that the paralysis was due to intramuscular changes. The spinal view was held by Remak and Erb, but the preponderance of opinion at present points to the peripheral nerves as the most likely origin, and even Remak himself admits the lack of convincing testimony in favor of the spinal origin. "The constancy of peripheral changes and the infrequency of noteworthy changes in the cord make the peripheral the more probable theory; but if it is correct, there is yet no explanation of the almost regular escape of certain muscles from the paralysis."<sup>1</sup>

With the hope of elucidating this interesting point, Dr. Warthin suggested that I make an attempt to corroborate the view which is known as Edinger's theory. He explains these peculiar saturnine paralyses as the result of excessive strain on certain groups of muscles. In this way, he accounts for the common wrist drop.

His conclusions are as follows:

(a) Of the forearm, the flexors (triceps, anconeus, extensors, biceps, brachialis anticus and supinator longus) possess a high degree of capacity for work; while the supinators are characterized by great mass, and are brought into play mainly in work of coarse and heavy nature, and not during fine manipulation.

(b) The muscles concerned in pronation are of small capacity for work, and are not called on for sustained work. As for the muscles acting on the wrist and hand, he concludes that the extensors (carpi radialis longior and brevior, carpi ulnaris and the extensors of the fingers) are powerful, and much exceed in capacity for work the flexors (flexor carpi radialis, flexor carpi ulnaris, the flexors of the fingers, etc.), but in all fine manual work, and especially where close grasping move-

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\* Submitted for publication July 10, 1913.

\* From the Pathological Laboratory, University of Michigan.

1. Edsall: Osler's Modern Medicine, i, 96.



ments enter into association with the flexors, external strain is put on them, while the flexors merely support their action. The extensor communis digitorum is the weakest of all the long finger muscles; its volume is hardly one-fourth that of the corresponding flexor, and while it acts only on the first of the phalanges, the flexors act on all three. In all fine work they are called on for heavy strain, especially the interossei and the lumbricales, but in harmony with the long flexors when grasping movements are performed. The small muscles of the fingers have nearly the same mass as the extensor communis, and in all fine movements or grasping efforts are taxed severely; but their play is under considerably more favorable physical relations than that of the extensors, while at times they are aided by the long flexors. The chief adductor muscle of the thumb (extensor metacarpi pollicis) is particularly powerful; the other extensors of the thumb are very weak and work under unfavorable physical conditions, but are supported in their action by the strong adductor muscle. The muscles of the abductor opponens and flexor brevis in the complicated work thrown on the thumb in manipulation are much exerted, so that the effects of over-exertion show themselves first in this region.

Thus Edinger maintains that the muscle-supply of the arm is designed for heavy work, the muscles of the finger and hand having to carry more work than can be expected of them from a consideration of their volume and physical action.

Teleky<sup>2</sup> reviews forty cases of paralysis with special reference to this theory. Thirteen were cases of the antibrachial type of D'jerine-Klumpke. In only one was the left hand affected, while in all the others except one, the right hand was affected. In it both hands suffered. These facts are significant in their relation to causation by employment.

The paralysis of the small muscles of the hand is rather characteristic in file cutters. As is known, these muscles are used predominately in this trade. Goadby and Legg<sup>3</sup> have frequently observed the decrease in size of the thumb and hypothenar eminences in lead rollers.

Of fourteen painters suffering from plumbism, Teleky<sup>2</sup> found three with paralysis of the right forearm only, while the remainder had both arms affected, but always more marked in the right than in the left. In some of these, the shoulder muscles were paralyzed, owing to the strain caused by raising the arms above the head, or lying on the back to paint the running gears of carriages.

Particularly interesting are the cases cited by Teleky in lead capsule polishers. These cases showed right-sided paralysis of the adductor brevis pollicis supplied by the median nerve, and partial paralysis of the

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2. Teleky: *Deutsch. Ztschr. f. Nervenheilk*, 1909, xxxvii.

3. Goadby and Legg: *Lead Poisoning and Lead Absorption*, Longmans & Co.

long extensors and of the extensor ossis metacarpi pollicis supplied by the radial nerve, and in one or two cases complete paralysis of the thenar muscles, and adductor, while the extensors of the fingers and wrists were only partially paralyzed. This unusual muscular involvement is undoubtedly the result of the peculiar movement necessary in polishing the capsules of bottles on a revolving spindle, involving especially the use of the opponens muscle.

Teleky reports a shoemaker who was poisoned with white lead. He developed not only paralysis of the adductors and extensors of the thumb, but also a paralysis of a lower extremity. He explains the latter by the exertion thrown on the adductor muscles of the thigh while holding the shoe.

In children affected with lead palsy the frequency of paraplegia has often been noticed. Both Oliver<sup>4</sup> and Goadby and Legg,<sup>3</sup> as well as Escherich and Variot<sup>5</sup> make mention of it.

I have to report as an original clinical observation, a painter, who, when plying his trade, had more climbing of ladders and mixing of paints to do than actual work with the brush. He developed muscular weakness and paraplegia some time before developing the wrist drop.

Goadby and Legg<sup>3</sup> have poisoned cats experimentally, and found that the quadriceps extensor muscles supplied by the anterior crural nerve was the first group affected, while soon after the lumbar group of spinal muscles was involved. This selection is significant, considering that these are the groups used in jumping movements.

These observers have also found hemorrhages in the nerve (anterior crural) and consider the minute hemorrhages resultant on the degeneration of the venioles to be of paramount importance in the pathology of lead poisoning.

From these observations from varied sources, one fact stands out rather strikingly — there is no necessary anatomical relationship of the paralysis in plumbism, but the functional relationship of involved muscles seems essential.

Gombault<sup>6</sup> sought to explain the paralysis of the hand and arm muscles by the absorption of lead through the skin, but this hypothesis is rather absurd when we come to account for the ocular or shoulder paralysis, or even that of the lower extremities.

Quoting from Alice Hamilton's article,<sup>7</sup> Labbe is authority for the statement that storage battery workers suffer from paralysis of the shoulder muscles. Bernhardt and Leichtentritt speak of the paralysis

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4. Oliver: "Occupational Pathology."

5. Escherich and Variot: *Jour. Am. Med. Assn.*, 1911, lvi, 1240. Quoted in Alice Hamilton's Article.

6. Gombault: *Arch. Physiol.*, 1873.

7. Hamilton, Alice: *Jour. Am. Med. Assn.*, 1911, lxi, 1240.

of the left fingers and thumb in file makers who grasp the chisel with the left hand.

Patch<sup>8</sup> relates a case of marked atrophy and paralysis of the shoulder muscles in a man who ran a "delicatessen." He was accustomed to do much lifting and first noticed that he was not able to lift as much as formerly. He presumably contracted the disease by substitution of a lead pipe in the connections of his soda fountain. C. H. Snover<sup>9</sup> of Randolph, N. Y., reports a case of a 16-year-old girl who sealed cans in a milk condensery. He says in part:

With arms extended, there was a marked wrist drop, especially on the right. The patient used the right hand to use the soldering iron. The left hand could be extended to bring it in line with the forearm, but no more, and this only with difficulty. No extension was possible in the right hand.

Since the fatigue basis has such logical clinical verification, Dr. Warthin thought it wise to ascertain what could be accomplished experimentally to validate this claim.

A. DETERMINING THE SUSCEPTIBILITY OF THE FROG TO NEUTRAL LEAD ACETATE.  
(FROGS 1 AND 2)

	Date	Amount of Lead Given	Notes
Frog 1..... Wt. 30 gm.	11/15/12	One c.c. sat. aqueous neutral lead acetate into dorsal lymph-sac.	11:30 a. m. Moderate stupor and diminution of reflexes.
	11 a. m.	All structures in right thigh ligated except the sciatic nerve. Frog pithed. Reflex irritability soon recovered.	12:00 m. Increase of stupor. Crossed reflexes negative. 1 p. m. Reflexes disappeared. 1:30 p. m. Death, as determined by cessation of circulation in the web of the foot. Reaction to weak induction shock. Stimulation of the right and left gastrocnemii, both directly, and by way of the sciatic nerve is positive.
Frog 2..... Wt. 27 gm.	11/15/12	Two c.c. neutral lead acetate into dorsal lymph-sac.	2:15, stupor and reflex diminution.
	2 p. m.	Same as above.	2:30 marked stupor. Cross reflexes negative. 3:00, increase of stupor. 3:45, death. Reaction to weak induction shock same as above.

8. Patch: Boston Med. and Surg. Jour., 1909, 653.

9. Snover, C. H.: Jour. Am. Med. Assn., 1911, Ivi, 1799; case report.

In these experiments, frogs were the animals selected, because they were most convenient for stimulation and because the results of fatigue could be accurately computed in a mechanical way. It was first necessary to determine their susceptibility to a soluble salt of lead, and also interesting to determine its sphere of action. To this end the Curare Experiment was performed with lead acetate. Briefly, this consists in ligation of all the structures of the thigh, excepting the sciatic nerve, and then the injection of the poison into the dorsal lymph-sac.

B. DETERMINING THE MINIMUM LETHAL DOSE OF NEUTRAL LEAD ACETATE IN FROGS. FROGS THREE TO EIGHT, INCLUSIVE.

SERIES ONE

	Date	Amt. of Lead Into Dorsal Lymph-Sac c.c.	Results
Frog 3 . . . . . Wt. 28 gm.	11/17/12 8 a. m.	0.25	Negative at any time.
Frog 4 . . . . .	11/17/12 8 a. m.	0.50	9 a. m., stupor and diminution of reflex, which gradually abated and frog finally recovered.
Frog 5 . . . . . Wt. 30 gm.	11/17/12 8 a. m.	0.75	9 a. m., stupor and reflex diminution. 10 a. m., stupor marked. Condition grew progressively worse until 3 p. m., when frog died.

SERIES TWO

Frog 6 . . . . . Wt. 27 gm.	11/18/12 8 a. m.	0.5	Same as 4
Frog 7 . . . . . Wt. 29 gm.	11/18/12 8 a. m.	0.6	11 a. m., slight stupor. 12 m., increase. 6 p. m., dead
Frog 8 . . . . . Wt. 30 gm.	11/18/12 8 a. m.	0.7	10 a. m., slight stupor. 11 a. m., marked stupor. 3 p. m., dead.

0.6 c.c. saturated aqueous solution neutral lead acetate is M. L. D. for 30 gm. frog; or 0.02 c.c. per gm.

Two c.c. saturated solution of lead acetate were so injected into a 25-gm. frog, which killed it in about two hours. The animal failed to respond to reflex stimulation, this failure being of gradual development. Direct application of the electrodes to the muscles provoked a marked response. Stimulation of the sciatic nerves also produced marked muscular contraction. At no time were any crossed reflexes observed. This experiment proves that the frog is susceptible to lead acetate, and that it does



## C. DETERMINING THE RESULTS OF FATIGUE PRODUCED BY THE DIRECT METHOD IN LEAD POISONED FROGS. FROG 9. WEIGHED 25 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
12 /2/12 5 p. m.	0.5	15"	10½	Stimulation applied to right gastrocnemius muscle; an electrode at either end.
12/ 3/12	...	15"	10½	Frog shows stupor.
12/ 4/12	...	...	....	Reacts poorly.
12/ 4/12	...	14"	10½	Stupor. Reacts poorly.
12/ 6/12	...	...	....	Frog normal again.
12/ 7/12	...	15"	10½	Induration at electrodal points.
12/ 8/12	...	13"	10½	
12/ 9/12	...	13"	10½	Stupor.
12/10/12	...	...	....	Frog dead.

## Reaction to Induction Shock:

Gastrocnemii, direct application:

Right negative to weak current; positive to strong current.

Left at 10½ negative; 9 cm. positive.

Sciatics: Positive at 10½. Right gastrocnemius shows 97.3 gm. millimeters of work; left, 112.4 gm. millimeters of work.

## FROG 10. CONTROL TO FROGS 9 TO 20. WEIGHT 28 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
12/ 2/12	...	15"	10½	Stimulation applied to right gastrocnemius an electrode at either end. Induration at electrodal points.
12/ 3/12	...	15"	10½	
12/ 4/12	...	15"	10½	
12/ 6/12	...	Rest	....	
12/ 7/12	...	13"	10½	
12/ 8/12	...	12"	10½	Frog killed.
12/ 9/12	...	14"	10½	
12/10/12	...	...	....	

## Reaction to Induction Shock:

Gastrocnemii, direct application:

Right at 10½ cm. weak; at 9 cm. strong.

Left at 12 cm. strong.

Right gastrocnemius, 132.6 gm. millimeters of work; left, 252.4 gm. millimeters.

not perceptibly affect the muscles directly, the end plates or the peripheral nerves. Since the animal's brain was destroyed by pithing, by exclusion we arrive at the fact that in the frog the spinal cord is the sphere of action of lead, given in doses sufficient to produce acute effects. (See Section A, Frogs 1 and 2. Protocol of Experiments.)

FROG 11. WEIGHT 30 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
12/ 4/12	0.35	12"	10	Direct Stimulation of right gastrocnemius.
12/ 5/12	...	13"	10	
12/ 6/12	...	12"	10	Moderate stupor.
12/ 7/12	...	12"	10	Moderate stupor.
12/ 8/12	...	12"	10	
12/ 9/12	...	12"	10	
12/10/12	...	...	....	Killed frog.

## Reaction to Induction Shock:

Gastrocnemii, direct application:

Right, 10 negative and 9½ negative; 8 cm. positive.

Left, 10 cm. negative; 9 cm. positive.

Sciatics: Positive at 10½ cm. Right gastrocnemius, 107 gm. millimeters of work; left, 117.5 gm. mil. of work.

FROG 12. WEIGHT 30 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
12/ 6/12	0.3	12"	10	Direct stimulation of right gastrocnemius.
12/ 7/12	...	12"	10	
12/ 8/12	...	12"	10	
12/ 9/12	...	12"	10	
12/11/12	...	12"	10	
12/12/12	...	12"	10	
12/13/12	...	12"	10	
12/14/12	...	12"	10	
12/15/12	...	12"	10	Killed frog.

## Reaction to Induction Shock:

Practically the same as in No. 11.

Work: Right gastrocnemius. 104.3 gm. mil.; left, 119.1 gm. mil.

A saturated aqueous solution of neutral lead acetate was used exclusively in all the experiments, because it is the most soluble salt. It was always injected into the dorsal lymph-sac. The weight of the frogs was always between 25 and 35 gm.

It was next necessary to determine the minimum lethal dose of lead acetate for these animals. (See Protocol of Experiments, Section B, Frogs 3 to 8, inclusive.) In Series I of three frogs, Frog 3 was given 0.25 c.c. of the lead solution, and the dose progressively increased by an increment of 0.25 c.c. in the remaining two. The first two frogs of the

## FROG 13. WEIGHT 25 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
12/ 9/12	...	13"	10	Direct stimulation of right gastrocnemius.
12/10/12	...	13"	10	
12/11/12	...	13"	10	
12/12/12	...	13"	10	
12/13/12	...	13"	10	
12/14/12	...	13"	10	
12/15/12	...	13"	10	
12/16/12	...	13"	10	
12/17/12	0.3	13"	10	
12/22/12	...	...	10	Frog killed.

## Reaction to Induction Shock:

Gastrocnemii, direct application:

Right, 11 cm. negative; 10 cm. negative; 9½ cm. positive.

Left, 11 cm. negative; 10 cm. positive.

Work: Right, 119.2 gm. mil; left, 173.8 gm. mil.

## FROG 14. WEIGHT 30 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
12/ 9/12	...	12"	10	Direct stimulation of right gastrocnemius.
12/10/12	...	12"	10	
12/11/12	...	12"	10	
12/12/12	...	12"	10	
12/13/12	...	12"	10	No symptoms.
12/14/12	...	12"	10	No symptoms.
12/15/12	...	12"	10	No symptoms.
12/16/12	...	12"	10	No symptoms.
12/17/12	0.35	12"	10	Killed frog.
12/22/12	...	...	....	

## Reaction to Induction Shock:

Gastrocnemii, direct application:

Right at 9 cm. positive; at 10 cm. negative.

Left at 11 cm. positive

Work: Gastrocnemius, right, 125.2 gm. mil.; left, 169.7 gm. mil.

## FROG 15. WEIGHT 32 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
12/ 9/12	...	12"	10	Direct stimulation of right gastrocnemius.
12/10/12	...	12"	10	
12/11/12	...	12"	10	
12/12/12	...	12"	10	
12/13/12	...	12"	10	Frog died; no result.

## FROG 16. WEIGHT 27 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
12/ 9/12	...	12"	10	Direct stimulation of right gastrocnemius.
12/10/12	...	12"	10	
12/11/12	...	12"	10	
12/12/12	...	12"	10	
12/13/12	...	12"	10	
12/14/12	...	12"	10	
12/15/12	...	12"	10	
12/16/12	...	12"	10	
12/17/12	0.4	12"	10	No symptoms. Killed frog.
12/22/12	...	...	....	

## Reaction to Induction Shock:

Gastrocnemii, direct application:

Right at 9 cm. positive; at 10 cm. negative.

Left at 12 cm. positive.

Work: Right gastrocnemius, 127; left, 178.4.

## FROG 17. WEIGHT 28 GM.

Date	Amount of Lead, c.c.	Stimulation Amount of	Position of Secondary Coil, cm.	Notes
12/ 9/12	...	12"	10	Direct stimulation of right gastrocnemius.
12/10/12	...	12"	10	
12/11/12	...	12"	10	
12/12/12	...	12"	10	
12/13/12	...	12"	10	
12/14/12	...	12"	10	
12/15/12	...	12"	10	
12/16/12	...	12"	10	
12/17/12	0.45	12"	10	Slight stupor. Recovered. Frog killed.
12/18/12	...	12"	10	
12/19/12	...	12"	10	
12/22/12	...	...	....	

Reaction to induction shock and work practically the same as in Frog 16.



series (Frogs 3 and 4) recovered from doses of 0.25 c.c. and 0.5 c.c. of lead, respectively. Number 5 died with a dose of 0.75 c.c. The frogs in Series II (Frogs 6, 7 and 8) received, respectively, 0.5, 0.6 and 0.7 c.c. of lead. Number 6 recovered and Nos. 7 and 8 died from the effects of the lead. Thus 0.6 c.c. is the M.L.D. of neutral lead acetate for a 30-gm. frog, or 0.02 c.c. per gram.

FROG 18. WEIGHT 33 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
12/ 9/12	...	12"	10	Direct stimulation of right gastrocnemius.
12/10/12	...	12"	10	
12/11/12	...	12"	10	
12/12/12	...	12"	10	
12/13/12	...	12"	10	
12/14/12	...	12"	10	
12/15/12	...	12"	10	
12/16/12	...	12"	10	
12/17/12	0.48	12"	10	
12/18/12	...	12"	10	
12/19/12	...	12"	10	Progressive stupor.
12/20/12	...	12"	10	Progressive stupor.
12/21/12	...	...	10	Frog killed.

Reaction work practically the same as in Frog 16.

FROG 19. WEIGHT 31 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
12/ 9/12	...	12"	10	Direct stimulation of right gastrocnemius.
12/10/12	...	12"	10	
12/11/12	...	12"	10	
12/12/12	...	12"	10	
12/13/12	...	12"	10	
12/14/12	...	12"	10	
12/15/12	...	12"	10	
12/16/12	...	12"	10	
12/17/12	0.5	12"	10	
12/18/12	...	12"	10	
12/19/12	...	...	....	Progressive stupor. Frog killed.

Reaction to even strong induction slight, and work practically *nil*.

In Section C of Experiment Protocol, it is seen that 12 frogs were used. With these the direct method of stimulation prevailed throughout. This method consisted in the direct application of the electrodes at either

end of the gastrocnemius muscle. To obtain a suitable place for the electrodes, the muscle was denuded over an area of approximately 6 mm. square. This method was open to the objection that after varying lengths of time, the muscle underwent changes deleterious to the success of the experiments, as it afterward proved. Petechiae, induration and occasional sloughing occurred at the electrode points. It is self-evident that the more serious change would disqualify the muscle, and presumably the slighter changes were without favorable influence.

## FROG 20. WEIGHT 30 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
12/ 9/12	...	12"	10	Direct stimulation of right gastrocnemius.
12/10/12	...	12"	10	
12/11/12	...	12"	10	
12/12/12	...	12"	10	
12/13/12	...	12"	10	
12/14/12	...	12"	10	
12/15/12	...	12"	10	
12/16/12	...	12"	10	
12/17/12	0.5	12"	10	Marked stupor. Frog killed.
12/18/12	...	12"	10	
12/19/12	...	...	....	

Reaction to induction shock negative, even to strong current. Work practically *nil*.

## D. DETERMINING THE RESULTS OF FATIGUE BY THE INDIRECT METHOD IN LEAD POISONED FROGS

## FROG 21. WEIGHT 28 CM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
1/ 5/13	...	12"	8-9	Left gastrocnemius fatigued by indirect method. No symptoms at any time.
1/ 6/13	...	12"	8-9	
1/ 7/13	...	12"	8-9	
1/ 8/13	...	12"	8-9	
1/ 9/13	...	12"	8-9	
1/10/13	...	12"	8-9	
1/11/13	...	12"	8-9	
1/12/13	...	12"	8-9	
1/13/13	0.125	12"	8-9	Frog killed.
1/17/13	...	...	...	

Reaction to Induction Shock:

All muscles react well at 12 cm.

Work: Right gastrocnemius, 265.4 gm. mil.; left, 27.8 gm. mil.

Recourse to Section C shows considerable variance in the energy of the right and left gastrocnemii of Frog 10. As this animal had no lead at any time, we are forced to the conclusion that the diminution in the force of contractile power of the right or stimulated muscle is mostly the result of its damage during fatigue. This is made quite manifest when we consult Section D, Frog 24 (control); here the disparity in muscular power is so slight as to be irrelevant.

FROG 22. WEIGHT 28 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
1/ 5/13	...	15"	8-9	Left gastrocnemius fatigued by indirect method. No symptoms at any time.
1/ 6/13	...	12"	8-9	
1/ 7/13	...	15"	8-9	
1/ 8/13	...	15"	8-9	
1/ 9/13	...	15"	8-9	
1/10/13	...	15"	8-9	
1/11/13	...	15"	8-9	
1/12/13	...	15"	8-9	
1/13/13	0.175	15"	8-9	
1/17/13	...	...	...	Frog died.

FROG 23

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
2/ 5/13	...	13"	8-9	Left gastrocnemius fatigued by indirect method.
2/ 6/13	...	13"	8-9	
2/ 7/13	...	13"	8-9	
2/ 8/13	...	13"	8-9	
2/ 9/13	...	13"	8-9	
2/10/13	...	13"	8-9	
2/11/13	...	13"	8-9	
2/12/13	...	13"	8-9	
2/13/13	...	13"	8-9	
2/14/13	0.125	13"	8-9	
2/17/13	...	13"	8-9	
2/18/13	...	13"	8-9	
2/24/13	...	...	..	
				Frog killed.

For curve of work see chart (Fig. 2).

Work: Right gastrocnemius, 276.7 gm. mil; left, 13 gm. mil.

Although in this series the fatigued muscle in the lead-poisoned frog showed considerable diminution in energy, the qualitative difference is not decisive when compared with the control frog. The gastrocnemii of

Nos. 9, 11, 13, 14 and 16 show, respectively, 97.3, 107, 119.2, 125.2 and 127 gm. millimeters of work as against 132 gm. millimeters for control Frog. 10. Since Frog 9 was not killed, post mortem changes can partially account for its decrease, particularly when the opposite leg showed a corresponding change.

FROG 24. NORMAL CONTROL. WEIGHT 32 GM.

Date	Amount of Lead, c.e.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
2/ 5/13	...	13"	8-9	Left gastrocnemius
2/ 6/13	...	13"	8-9	stimulated by indirect
2/ 7/13	...	13"	8-9	method.
2/ 8/13	...	13"	8-9	No symptoms.
2/ 9/13	...	13"	8-9	No symptoms.
2/10/13	...	13"	8-9	No symptoms.
2/11/13	...	13"	8-9	No symptoms.
2/12/13	...	13"	8-9	No symptoms.
2/13/13	...	13"	8-9	No symptoms.
2/14/13	...	13"	8-9	No symptoms.
2/17/13	...	13"	8-9	No symptoms.
2/18/13	...	13"	8-9	No symptoms.
2/24/12	...	...	...	Frog killed.

For results, see curve and plot (Fig. 3).

Work: Right gastrocnemius, 287 gm. mil.; left, 274.5 gm. mil.

FROG 25. WEIGHT 33 GM.

Date	Amount of Lead, c.e.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
2/ 6/13	...	13"	8-9	Left gastrocnemius
2/ 7/13	...	13"	8-9	stimulated by indirect
2/ 8/13	...	13"	8-9	method.
2/ 9/13	...	13"	8-9	No symptoms.
2/12/13	...	13"	8-9	No symptoms.
2/13/13	...	13"	8-9	No symptoms.
2/14/13	...	13"	8-9	No symptoms.
2/15/13	...	13"	8-9	No symptoms.
2/16/13	0.175	13"	8-9	No symptoms.
2/20/13	...	...	...	Frog killed.

Reaction to Induction Shock:

Both gastrocnemii react well at 12 cm.

Work: Right gastrocnemius, 270.5 gm. mil.; left, 39.3 gm. mil.

An analysis of Section D gives an entirely different view. Here most of the results are clear and decisive. There can be no question that the disparity in the contractile power of the muscles of Frogs 21, 23, 25 and 26 is not due to the lead, except as the fatigue facilitates the pathological changes which characterize it.



By reference to the plotted curve (Fig. 1) representing the experiment with Frog 23, it is obvious that its right gastrocnemius was able to do 276.7 gm. millimeters of work. The left, or fatigued gastrocnemius, was able to perform but a scant 13 gm. millimeters of work. It is self-evident that the former is able to do 21.24 times the work done by the latter.

FROG 26. WEIGHT 31 GM.

Date	Amount of Lead, c.c.	Stimulation Amount of	Position of Secondary Coil, cm.	Notes
2/ 7/13	...	14"	8-9	Left gastrocnemius fatigued by indirect method.
2/ 8/13	...	14"	8-9	
2/ 9/13	...	14"	8-9	
2/10/13	...	14"	8-9	
2/11/13	...	14"	8-9	
2/12/13	...	14"	8-9	
2/13/13	...	14"	8-9	
2/14/13	...	14"	8-9	Very slight reflex diminution. Frog recovered. Frog killed.
2/15/13	0.2	14"	8-9	
2/16/13	...	14"	8-9	
2/21/13	...	...	...	

Reaction to Induction Shock:

Both gastrocnemii react well at 12 cm.

Work: Right gastrocnemius, 221.7 gm. mil.; left, 17.8 gm. mil.

FROG 27. WEIGHT 31 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
2/18/13	...	12"	8-9	Left gastrocnemius fatigued by indirect method.
2/19/13	...	12"	8-9	
2/21/13	...	12"	8-9	
2/22/13	...	12"	8-9	
2/23/13	...	12"	8-9	
2/25/13	...	12"	8-9	
2/26/13	...	12"	8-9	
2/27/13	...	12"	8-9	Slight decrease in reflexes and stupor. Frog killed.
2/28/13	0.225	12"	8-9	
3/ 2/13	...	12"	8-9	
3/ 4/13	...	12"	8-9	

Reaction to Induction Shock:

Both react well at 12 cm.

Work: Right gastrocnemius, 245.3 gm. mil.; left gastrocnemius, 17.9 gm. mil.

In order to eliminate the effect of the stimulation and lead separately, a normal frog of the same weight as No. 23 was given the same amount

of stimulation without the lead. This is Frog 24 (Fig. 2). By calculation it is seen that the right gastrocnemius has 287 gm. millimeters of work, while the left or fatigued gastrocnemius has 274.5 gm. millimeters. This leaves a difference of 12.5 gm. millimeters to account for the effect of fatigue in a normal frog, and 10.3 gm. millimeters for the effect of the lead. These are insignificant differences, particularly the 10.3 gm. millimeters which could be physiologic in character.

FROG 28. WEIGHT 30 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
2/16/13	...	13"	8-9	Left gastrocnemius fatigued by indirect method.
2/17/13	...	13"	8-9	
2/18/13	...	13"	8-9	
2/19/13	...	13"	8-9	
2/20/13	...	13"	8-9	
2/21/13	...	13"	8-9	
2/24/13	...	13"	8-9	
2/25/13	...	13"	8-9	
2/26/13	0.25	13"	8-9	Frog killed.
3/ 2/13	...	13"	8-9	

## Reaction to Induction Shock:

Both gastrocnemii react well at 12 cm.

Work: Right gastrocnemius, 195.6 gm. mil.; left, 19.8 gm. mil.

FROG 29. WEIGHT 33 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
3/ 3/13	...	12"	8-9	Left gastrocnemius fatigued by indirect method.
3/ 4/13	...	12"	8-9	
3/ 5/13	...	12"	8-9	
3/ 6/13	...	12"	8-9	
3/ 7/13	...	12"	8-9	
3/ 8/13	...	12"	8-9	
3/ 9/13	...	12"	8-9	
3/10/13	...	12"	8-9	
3/11/13	...	12"	8-9	Slight stupor. Recovered fully.
3/12/13	0.3	12"	8-9	
3/14/13	...	12"	8-9	
3/15/13	...	12"	8-9	
3/16/13	...	12"	8-9	Frog killed.
3/16/13	...	12"	8-9	
3/20/13	...	...	...	

## Reaction to Induction Shock:

Both gastrocnemii react well at 12 cm.

Work: Right gastrocnemius, 203.6 gm. mil.; left, 21.7 gm. mil.

FROG 30. WEIGHT 32 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
3/ 8/13	...	12"	8-9	Left gastrocnemius fatigued by indirect method.
3/ 9/13	...	12"	8-9	
3/10/13	...	12"	8-9	
3/11/13	...	12"	8-9	
3/12/13	...	12"	8-9	
3/13/13	...	12"	8-9	
3/14/13	...	12"	8-9	
3/15/13	...	12"	8-9	
3/16/13	...	12"	8-9	
3/17/13	0.35	12"	8-9	Well developed stupor followed Pb. injection. Frog recovered only partially; at times fairly irritable; at other times listless.
3/20/13	...	12"	8-9	
3/21/13	...	...	...	

## Reaction to Induction Shock:

## Direct application:

Right at 12 negative; at 11.5 positive.

Left at 11 negative; at 10.5 positive.

Work: Right gastrocnemius, 183; left, 21.2.

FROG 31. WEIGHT 29 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
3/15/13	...	12"	8-9	Left gastrocnemius fatigued by indirect method.
3/16/13	...	12"	8-9	
3/17/13	...	12"	8-9	
3/18/13	...	12"	8-9	
3/19/13	...	12"	8-9	
3/20/13	...	12"	8-9	
3/21/13	...	12"	8-9	
3/22/13	...	12"	8-9	
3/23/13	...	12"	8-9	
3/24/13	0.45	12"	8-9	Marked stupor and reflex diminution following Pb. injection. Recovery only slight, and then seemed to grow progressively worse.
3/28/13	...	...	...	

## Reaction to Induction Shock:

## Direct application:

Right gastrocnemius at 10 negative; at 9 positive.

Left gastrocnemius at 8 negative, at 7 positive.

Work: Right gastrocnemius, 141.3 gm. mil.; left, 15.4 gm. mil.

Frogs 29, 30, 31 and 32 show the effect of increasing dosage by producing stupor and diminution of reflexes. The confusion in the results of the quantitative work is also noted. Although the fatigued muscle shows a very marked decrease in energy, the unfatigued muscle is also affected so markedly that one is at a loss to know how much to attribute to fatigue and how much to the lead itself. This confusion is averted in the frogs referred to above.

In this work, two methods of fatigue were used. First, a direct application of the electrodes to the muscles, and second, an electrical irritation of the skin which produced a muscular contraction resulting from the frog's attempt to get away from the irritation. This was accomplished by an apparatus which I devised for the purpose, the details of which appear later.

FROG 32. WEIGHT 32 GM.

Date	Amount of Lead, c.c.	Amount of Stimulation	Position of Secondary Coil, cm.	Notes
3/20/13	...	12"	8-9	Left gastrocnemius fatigued by indirect method.
3/21/13	...	12"	8-9	
3/22/13	...	12"	8-9	
3/23/13	...	12"	8-9	
3/24/13	...	12"	8-9	
3/25/13	...	12"	8-9	
3/27/13	...	12"	8-9	
3/28/13	...	12"	8-9	
3/29/13	...	12"	8-9	
3/30/13	0.53	12"	8-9	Marked stupor which grew progressively worse. Reflexes also diminished markedly. Frog will probably not recover.
3/31/13	...	...	...	
4/3 /13	...	...	...	

## Reaction to Induction Shock:

## Direct application:

Right gastrocnemius at 7 negative; at 6 positive.

Left gastrocnemius at 4 negative; at 3 positive.

Work: Right gastrocnemius, 75.4 gm. mil.; left, practically *nil*.

The first method was fairly successful in the gross, but was a complete failure when accurate methods were used. This is clearly shown by the experiments in Section C of the Protocol of Experiments. The reasons for the inefficiency of this method have already been explained. The abandonment of this method resulted in the adoption of another, which makes use of an apparatus which the subjoined diagram will render obvious (Fig. 3).



Tension is put on the left leg of the frog by anchoring it to the binding post P. A piece of twine is used which is about an inch long. It is not well to have it longer, as too much freedom of motion obtains. Some suitable electrode is placed adjacent to the tendon of the gastrocnemius at A, the height of the electrode being regulated by a piece of tin-foil which supports it at T F.

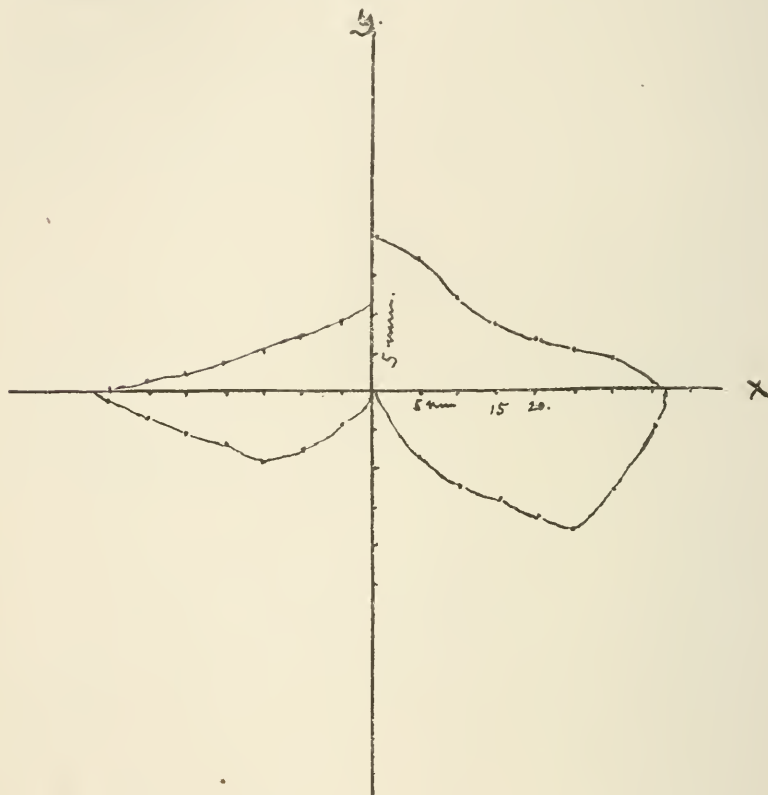


Fig. 1.—Explanation of Plotted Curves. (Frog 23.) The curves represent the type of results derived from the indirect form of stimulation in a lead frog. The abscissa and ordinate are ruled off in spaces of 5 mm. each. To the right of the ordinate Y is represented the curve of lift and curve of work of the right gastrocnemius. The former is above the abscissa, and the latter below. To the left of the ordinate is represented the curves of lift and work of the left gastrocnemius. The disparity shown between the two curves of work and lift is not absolute, as those for the left leg are magnified ten times.

One electrode is fastened to the front leg of the frog by a curved copper wire, and the current is closed by the contact of the electrode with the tendon of the gastrocnemius. The resulting irritation causes a sudden contraction of the leg and thigh muscles, which break the circuit by the withdrawal of the leg from the electrode. The gastrocnemius is invariably contracted by this movement, as can be evidenced by palpation.

The break in the circuit almost instantly permits the leg to drift back on the point of the electrode when the contraction is repeated. In a normal 25-gm. frog, this rate may run from three to four per second, but it is not long before the rate becomes rapidly decreased to one or two per second.

It is entirely self-evident that the greatest advantage accruing from this simple device is the one which makes use of fatigue of muscles by mechanical means, even though the induction coil is used as the exciting factor.

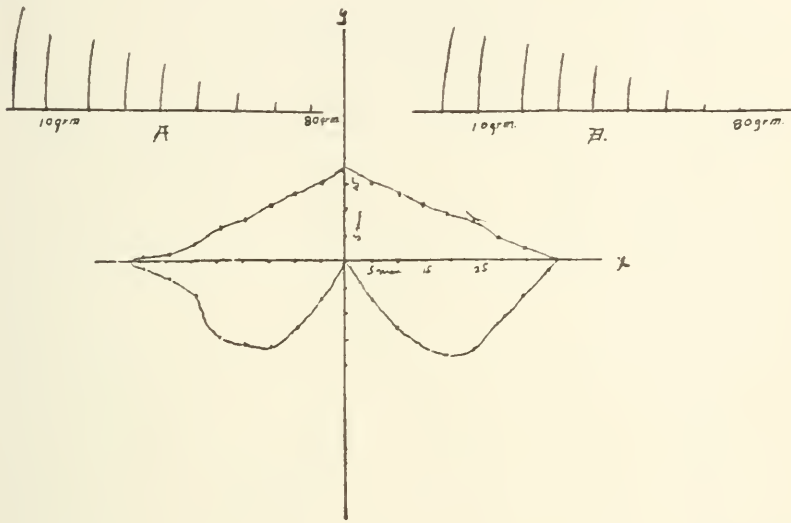


Fig. 2.—Explanation of Plotted Curve. (Frog 24.) The curve represents the results obtained by the indirect form of stimulation in a control frog which had received no lead. The curves to the right of the abscissa represent the right leg, and those to the left, the left leg. There is no magnification of any curve on either side. Tracings A and B represent the excursion of the left and right gastrocnemii muscles, respectively, under stimulus of a weak induction shock, and weighted with loads of from 10 to 80 grams.

This method besides being admirably adapted to our purpose, in that it imitates fairly perfectly the actual contraction of human muscles in doing work, requires no additional apparatus. The pure electrical form of stimulation, even when perfect, is open to the objection that it does not parallel closely the mechanical form used by man in his labor. Its applicability to our proposition is well illustrated by the fact that muscles of a normal frog do not lose perceptibly in power when stimulated in this way, when sufficient time is given for recovery from the fatigue induced.

The experiments showed the importance of the administration of a subphysiological dose of lead. Good results were almost always obtained when the doses were not so large as to produce stupor and marked dimin-

ution of reflexes to mechanical and electrical stimulation. In case this caution is not observed, the non-stimulated as well as the stimulated muscles show loss of power.

As in all work of this nature, a terminal recovery interval should be observed. In order to determine whether the loss of power in a muscle is permanent, the effects of ordinary fatigue should be permitted to disappear. In muscles fatigued only once a day, forty-eight to seventy-two hours suffice admirably for such restoration. Possibly less time would do, but a generous margin was left so it would not be possible to account for the loss of power in the lead frog from the effects of the stimulation.

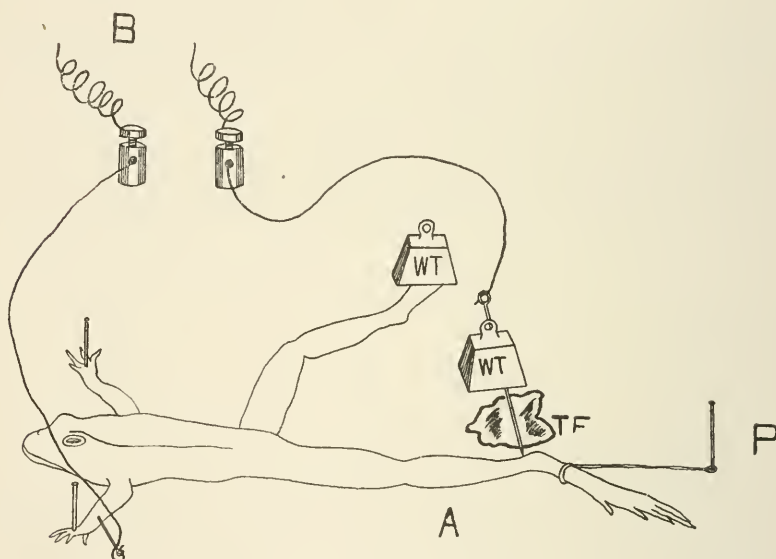


Fig. 3.—Diagram representing the indirect method of inducing fatigue of muscle. For explanation see text.

#### SUMMARY

1. That the quantitative differences between the amount of work done by the fatigued and non-fatigued leg in a lead-poisoned frog is not conclusive when the direct application of the electric current is used for muscle fatigue.

2. No conclusive quantitative differences are observed where large enough doses of the metal are given to produce stupor and marked diminution of reflexes.

3. That these experiments, as worked out by the indirect method of fatigue production, are conclusive evidence of the validity of Edinger's

contention, viz., that lead in itself has no predilection for any group of muscles except as that choice is made possible by their over-use. Fatigue is, therefore, the main factor in the localization of lead palsies.

The Harvard inductorium, manufactured by the Harvard Apparatus Co., was used in all the experiments. The battery was a single Columbia dry cell.

Acknowledgement is made to the Department of Physiology, to Dr. Lombard, Dr. Cope and Mr. F. T. Munson for apparatus loaned me; and to Dr. Warthin for the suggestion of the problem and his control of the investigation.



## ON THE REINSPIRATION OF EXPIRED AIR \*

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I desire to record a phenomenon and to discuss its significance.

The phenomenon is, the immediate re-inspiration of a portion of our expired air. This occurs quite commonly — so commonly, in fact, that it is an accompaniment of respiration during the major part of the lives of many people, and during a large part of the lives of practically all.

The observation of this phenomenon is not new. Lehmann<sup>1</sup> and Heymann<sup>2</sup> have each reported a small group of experiments, in which they determined the carbon dioxide of the inspired air, compared this with the carbon dioxide of the surrounding air, and from the difference computed the proportion of the breath which was re-inspired. The proportion varied greatly. It was sometimes more than 6 per cent.; it dropped to zero in the open air and in a breeze of 3 meters per second.

A few years ago this subject attracted my attention in connection with certain studies of ventilation. The experiments of Lehmann and Heymann demonstrated little except that re-inspiration may and does occur. It seemed desirable to carry the observations further. As opportunity arose this has been done, and an attempt has been made to determine some of the factors controlling this phenomenon. The work has included the analysis of about 900 samples of inspired air.

### METHODS

In order to find what proportion of the expired air re-enters the respiratory tract with the succeeding inspiration, it is necessary to collect samples of the inspired air as it enters the nasal passages and subject them to analysis. The only difficulty in collecting the samples arises through the possibility of including a portion of the expired air as it *leaves* the nasal passages. This is likely to occur if the taking of the sample is not accurately timed to the inspiratory period; collection must begin after inspiration is established and must end before expiration has begun.

Experiments such as these are more readily carried out on oneself than on a second person, because of the easier identification of the

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\* Submitted for publication July 15, 1913.

1. Lehmann: Der Kohlensäuregehalt der Inspirationsluft im Freien und im Zimmer. Arch. f. Hyg., 1899, xxxiv, 315.

2. Heymann: Ueber den Einfluss wieder eingeathmeter Expirationsluft auf die Kohlensäure-Abgabe. Ztschr. f. Hyg., 1905, xlix, 388.

respiratory periods. With a little practice one may become perfectly conscious of the beginning and end of inspiration; and with a little more practice may learn to bring the inlet tube of a bellows into the nasal orifice at the proper moment and to remove it at the proper moment, using one hand meanwhile to manipulate the bellows. By arranging for an air current that will immediately carry away all of the expired air, a series of controls may be run that will show clearly when the technic is mastered. The observations herewith recorded were made by the author on himself. Those made by the experimenters above referred to were made on a second person.

After various trials of more or less complicated apparatus, a very simple plan of collecting samples was adopted. The ordinary Paquelin cautery bulb was found to answer very well for this purpose after a little alteration. The only necessary alterations are a long rubber tube with



Fig. 1.—Apparatus used in collecting samples of air. A, intake tube; B, bellows; B', the elastic bag; O, outlet tube.

a glass tip attached to the bellows intake, and a pinch-cock applied to the outlet tube from the elastic bag. A somewhat larger and heavier bag than that ordinarily supplied with the cautery was used, but the arrangement was essentially the same. The accompanying photograph (Fig. 1) shows the apparatus used, and the case for collecting and carrying samples and records. A is the intake tube, B is the bellows, B' the elastic bag and O the outlet tube.

After inspiration is well established the glass inlet tube is brought just within the nasal orifice — or between the parted lips during mouth breathing — and the bellows given two or three quick compressions. The inlet tube is removed during expiration and is again brought into place

during the succeeding inspiration. This is repeated during eight or ten breaths, or until about 1,000 cubic centimeters of air have been collected in the bag. The outlet tube is then thrust to the bottom of a two-ounce bottle and the air run through it by releasing the pinch-cock. This leaves the sample of air in the bottle. On withdrawal of the tube a waxed glass stopper is inserted and the seal made perfect. If the bottle is clean and dry there will be no change in the air it contains for a fortnight or more. The samples are then transferred under a saturated solution of sodium chlorid to a Petterson-Palmquist apparatus and analyzed for carbon dioxid. The details of the various steps, the necessary precautions and the accuracy of the method have been previously discussed.<sup>3</sup>

At the time the samples of inspired air are collected, samples of the surrounding air must also be taken. The amount of immediate re inspiration is measured by the difference in the carbon dioxid content of these two. In the accompanying charts this difference is shown directly, and the proportion of  $\text{CO}_2$  in the air is expressed in ten-thousandths of the whole volume. In the text, re inspiration is referred to in terms of per cent.; that is, the proportion of expired air which is mixed with the inspired air and which is therefore reinhaled after it has been once expelled. It may be assumed that for every 1.5 per 10,000 difference between the  $\text{CO}_2$  of the inspired air and of the surrounding air, 1 per cent. of the expired air is being re inspired. This is derived by a very simple computation, based on the fact that the expired air contains about 450 volumes of  $\text{CO}_2$  in 10,000 volumes of air. It will of course vary a little from this figure, which is an average, but the variation will be relatively small and will affect the computation only slightly.

It should be remembered that the normal air contains 3.5 to 4 parts of  $\text{CO}_2$  per 10,000. All above this is contamination, the result of respiration, burning lights, or other chemical processes. In the majority of instances all  $\text{CO}_2$  above 4 per 10,000 represents respiratory contamination; and in a broad sense all  $\text{CO}_2$  above 4 per 10,000 in the inspired air generally represents re inspiration. But for our present purpose we shall consider only the  $\text{CO}_2$  which is in excess of the amount contained in the general surrounding air; for it is this portion only which represents that strictly local contamination which leads to the immediate re inspiration of our exhalations now under discussion.

#### RESULTS

It was soon noticed that, under apparently fixed conditions, repeated observations of the inspired air would always show considerable variation

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3. Crowder: A Study of the Ventilation of Sleeping Cars, *THE ARCHIVES INT. MED.*, 1911, vii, 85.

in the  $\text{CO}_2$  content. All experiments were therefore carried out with serial tests, including from 4 to 24 successive samples, in order to arrive at averages. The individual observations of such series can be made at about one minute intervals when one is standing or sitting. They will fall a little farther apart when lying down, or under any circumstances which occasion interference with the easy manipulation of the apparatus and the records.

It proved desirable to alter the surrounding conditions, especially the amount of ventilation, at the same time and place, or to induce artificial air currents, and observe the effects on the phenomenon under investigation by running parallel series of tests. The effect of changes of position, body motion, different types of breathing, different temperatures and different degrees of contamination of the surrounding air were also investigated; and finally, the question of whether or not re-inspiration takes place in such half-sheltered places as the sleeping-porch and in the open air. The results will follow in order. Unless otherwise specified, nasal breathing is referred to.

#### THE AIR ABOUT THE FACE

If the conditions are such that re-inspiration can take place the air about the face will necessarily be found to contain more  $\text{CO}_2$  than the surrounding air. It is on the fact that the expired air is not immediately removed or disseminated that re-inspiration depends. That it remains about the face for an appreciable interval, is easily demonstrated. This is illustrated in Figure 2.

The heavy horizontal line in this, as in succeeding charts, represents the  $\text{CO}_2$  in the general air of the room. It was constant to within half a point of 4.5 per 10,000. This half a point in 10,000, or 1 in 20,000, may be considered as about the maximum necessary error of the determinations. The series of samples represented by the upper curve, marked *A*, were taken close to the nose while holding the breath at the end of normal expiration; they contain an average of 3.8 per cent. of expired air. Those represented by the lower curve, marked *B*, were taken at the same place while holding the breath at the end of normal inspiration; they contain an average of 0.9 per cent. of expired air. The time occupied in collecting each of these samples was about five seconds. Those represented by the middle curve, marked *C*, were taken from within the nasal orifice during inspiration; they, therefore, show the contamination of the inspired air, which averaged 7.5 more  $\text{CO}_2$  per 10,000 than was contained in the air of the room. This excess of  $\text{CO}_2$  represents 1.7 per cent. of expired air; we therefore have for this series 1.7 per cent. of re-inspiration. The average for each series is shown by the short horizontal line at the right. This experiment was carried out while sitting in



a well-ventilated room of 3,000 cubic feet capacity, having three windows and three doors. The temperature was 73 F.

A little variation of the last experiment will show that this local contamination reaches a considerable distance below the face, and that it decreases with increasing distance. Figure 3 illustrates some of the results with such a variation. At *A* is shown a series of samples taken close to the body and 6 to 8 inches below the nose during expiration;

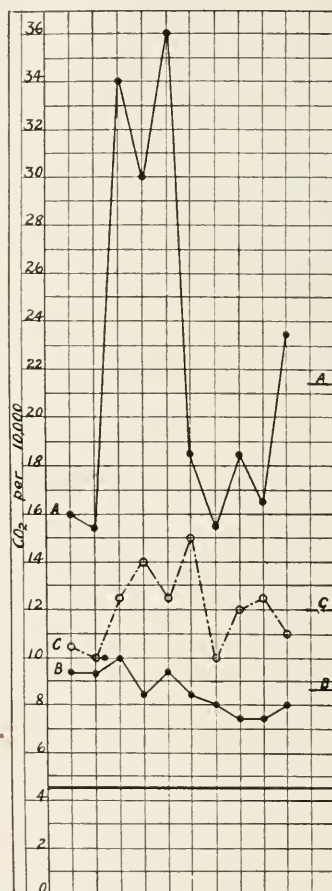


Fig. 2.—(Exper. 22). Showing contamination of the air about the face while holding the breath after expiration (*A*), after inspiration (*B*), and of the inspired air (*C*).

they contain an average of 4.6 per cent. of expired air. At *B* is shown a series taken 12 to 15 inches below the nose during expiration, and they contain 2.8 per cent. of expired air. At *C* the samples were taken 6 to 8 inches below the nose during inspiration, and contain 0.7 per cent. of expired air. At *D* the samples were taken 12 to 15 inches below the nose

during inspiration, and the contamination amounts to only 0.1 per cent. of expired air. These tests were made in the same room as the above, when there was a measured air-supply in excess of 15,000 cubic feet per hour entering through an open transom. The temperature was 66 F.

The conditions noted in Figures 2 and 3 are fundamental to our investigation. The contamination may be traced further; it tends to disappear rapidly, both in the time and space relation. The general facts are not particularly new or strange. They may be roughly determined by any one who will watch the course of smoke blown through the nostrils.

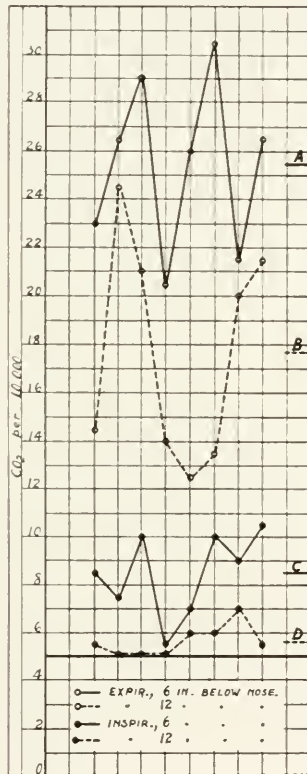


Fig. 3.—(Exps. 8 and 9). Showing contamination of the air 6 to 8 inches and 12 to 15 inches below the nose during expiration (A and B), and at the same places during inspiration (C and D).

#### THE EFFECT OF TEMPERATURE

It has been sometimes stated before scientific societies that when the surrounding air is cool — below 60 F. or thereabouts — the expired air will rise out of the breathing zone on account of its lower specific gravity, and reinspection will not take place. The results shown in the last chart would seem to cast a doubt on the theoretical correctness of this

view. Those making the statement have failed to consider the downward propulsion of the expired air from the nares, that it takes on a cone-shaped expansion, a part of which lies quite close to the body, and that convection currents tend to carry this part upward over the face. Inspiration succeeds expiration almost immediately; any upward current would therefore have to be very rapid in order to carry this air away before the latter process begins. Convection currents caused by the heat of the body, instead of being rapid, are just the opposite.

That reinspiration undoubtedly does take place at temperatures much below 60 F. will be seen from the two series of observations illustrated in Figure 4. That at the left was made in a large room of 16,000 cubic feet, with windows on three sides. It had a temperature of 50 F., and

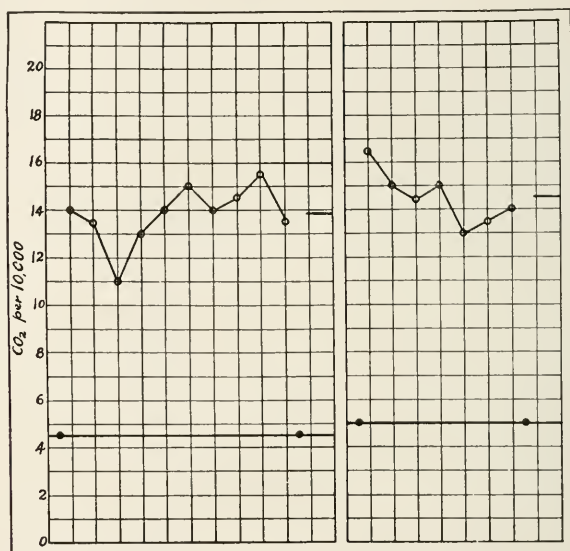


Fig. 4.—(Exps. 24 and 27.) Showing the inspired air while standing in a room of 16,000 cubic feet at 50 F. (at the left), and in a room of 1,500 cubic feet at 43 F. (at the right).

the reinspiration averaged 2.1 per cent. That at the right was made in a small room of 1,500 cubic feet. The temperature was 43 F. and the reinspiration also averaged 2.1 per cent.

If a low temperature has any effect it tends to increase reinspiration. Within certain limits I believe that it does this, though I am not prepared to show that it has any constantly appreciable effect. Figure 5 shows an attempt to make the comparison in a cold and in a warm air with otherwise uniform conditions. The experiment was carried out in a small bedroom of some 1,200 cubic feet capacity. The windows and doors were closed. All samples were taken while standing perfectly free,

about the middle of the room. Each series runs through about half an hour's time. For that shown on the left the temperature was 34 F. and the reinspiration amounts to 2.3 per cent. For that shown at the right the temperature was 70 F. and the reinspiration amounts to 2 per cent. The former was taken late at night, the latter late the next morning. The changing  $\text{CO}_2$  in the general air of the room may be accounted for by the presence of the experimenter; and the difference in the  $\text{CO}_2$  of this air for the two series by pure air entering from without through the window crevices in the first, whereas in the last the currents were reversed and air was entering from the hallway after being already slightly contaminated. In each instance the room had been unoccupied before the beginning of the experiment.

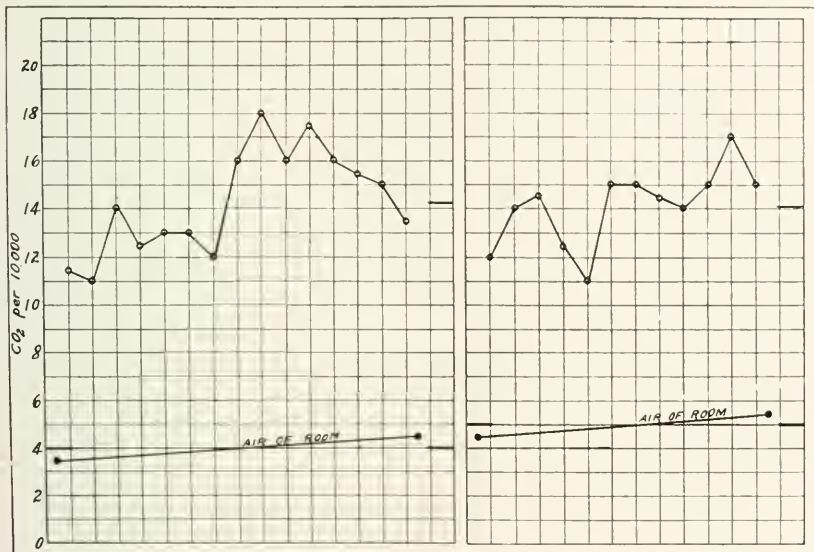


Fig. 5.—(Exp. 23). Showing the inspired air in a room with a temperature of 34 F. (on the left), and in the same room with a temperature of 70 F. (on the right), all other conditions remaining essentially the same.

#### THE EFFECT OF VENTILATION CURRENTS

It is reasonable to suppose that the greater the volume of air supplied to a room the less evident will be the phenomenon of reinspiration, because of the greater motion imparted to the air of the confined space. In a measure, this supposition is correct; but it does not follow that a large air-supply to a room will entirely prevent the immediate reinspiration of expired air by the occupants. There are a number of factors determining the limits of air-supply which will be found of too much importance to permit of a sufficient ventilation to control the matter entirely, or even to limit it in a very marked degree.



The results of an experiment carried out in a small bedroom of about 1,000 cubic feet, and also at the low temperature of 43 F., are illustrated in Figure 6. The room has one window and two doors. A strong wind was blowing without. The series shown at the left was taken with closed doors and window; in that at the right the window was raised so as to give one square foot of opening, and admitted air at the rate of 16,500 cubic feet per hour—enough to accomplish in excess of sixteen complete changes each hour. In the first, the re-inspiration amounts to 1.9 per cent.; in the second it fell to only 1.4 per cent. Most of the samples of air were taken while standing, a few while sitting on the edge of the bed, which was about 6 feet from the window. It is probable that there is a slight error in determining the  $\text{CO}_2$  in the air of the room for the

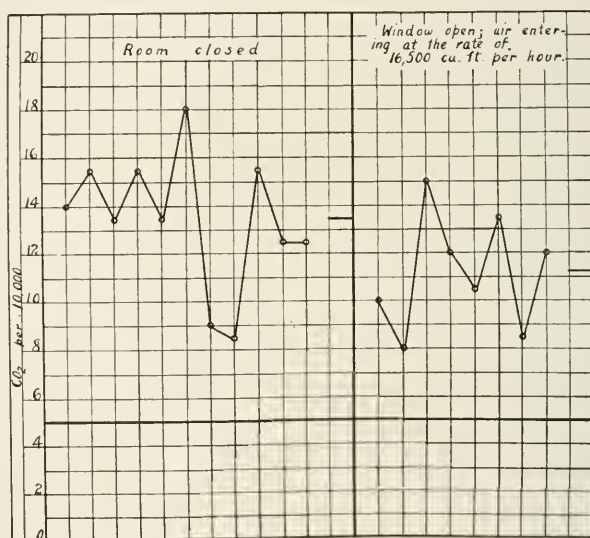


Fig. 6.—(Exp. 4). • Showing the inspired air in a room with a small air supply and in the same room with a large air supply.

second series, and that the base line should be dropped to 4, which would make the difference between the two groups still less striking.

The difference produced by a large air-supply, or even by a moderate one, is, in many instances, more marked than in the above experiment. When one sits or stands directly between the inlet and the outlet, and the air-supply is large, the proportion of the breath which is re-inspired may drop to nearly nothing. Two experiments made under these conditions in a room of 3,000 cubic feet with a temperature of 70 F. are shown in Figure 7. In each instance the air-supply came in through a door from the adjacent hallway and left through a window diagonally opposite. It was measured at the exit. The samples of inspired air were taken while

sitting directly in the line between door and window, and twice as far from the former as from the latter. In the curve marked *A* the air-supply was at the rate of 28,000 cubic feet per hour; in the curve marked *B* it was at the rate of 63,000 cubic feet per hour. The average reinspection is 1.1 per cent. for *A*, and 0.6 per cent. for *B*. The base line representing the  $\text{CO}_2$  in the general air of the room was not the same in these two instances, but in the chart the curves are adjusted to a common base of 5, in order to make a clear and accurate comparison.

On another occasion the same room was found to be receiving 324,000 cubic feet of air per hour through the open door. This was leaving through the open window as before. It was possible in this instance to measure by the anemometer the air current in the position occupied for

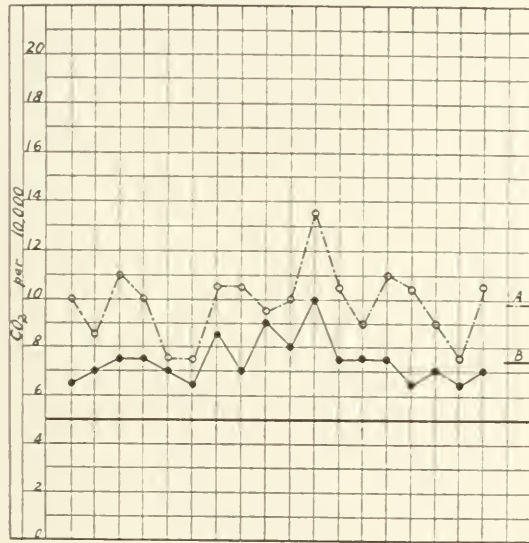


Fig. 7.—(Exps. 18 and 21). Showing the inspired air while sitting directly between inlet and outlet of a room of 3,000 cubic feet, ventilated by 28,000 cubic feet of air per hour (*A*), and by 63,000 cubic feet per hour (*B*).

the tests. This was found to be about 120 feet per minute. The negative results of the reinspection test are shown in the curve marked *A* in Figure 8. But on moving about 6 feet out of the direct line between the door and the window the result was changed to a reinspection of 0.6 per cent., as can be seen in the curve marked *B* in the same chart. In this latter position the anemometer moved irregularly, sometimes forward and sometimes backward. The amount of air being supplied during this experiment is of course much larger than can ordinarily be used to ventilate a room of this size, whatever the method of ventilation employed.

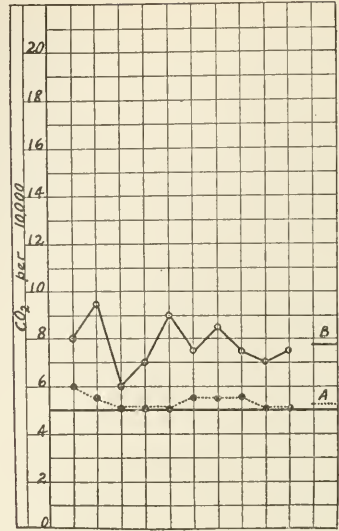


Fig. 8.—(Exper. 20). Showing the absence of reInspiration while sitting directly between inlet and outlet of a room of 3,000 cubic feet, ventilated by 324,000 cubic feet of air per hour (A), and its presence when sitting 6 feet out of the direct line (B).

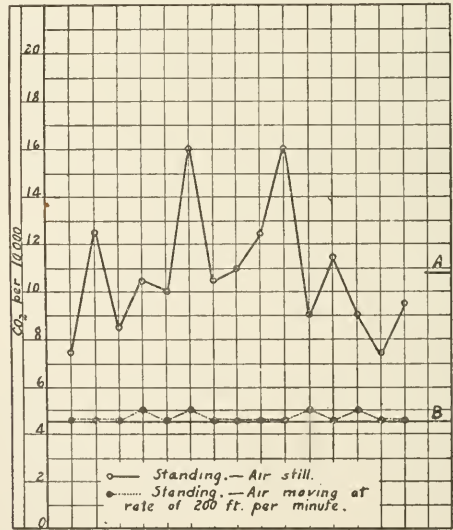


Fig. 9.—(Exper. 12). Showing that there is no reInspiration when facing a breeze of 200 feet per minute (B), contrasted with 1.4 per cent. reInspiration when the air is relatively still (A).

By the employment of properly directed artificial currents it is quite easy to prevent reinspiration entirely. This may be readily accomplished by means of an electric fan, provided the face is free to receive the breeze. If there is obstruction to the free flow of the current of air, the results may be quite different, as will be demonstrated later. Figure 9 shows very well what happens in a fan current moving at the rate of 200 feet per minute when there is no obstruction (curve *B*), and also what happens in the same place when the fan is shut off (curve *A*). Such an experiment also serves well as a control of the technic, which was identical for the two series. The room in which it was made contains 2,500 cubic feet and had a temperature of 68 F.

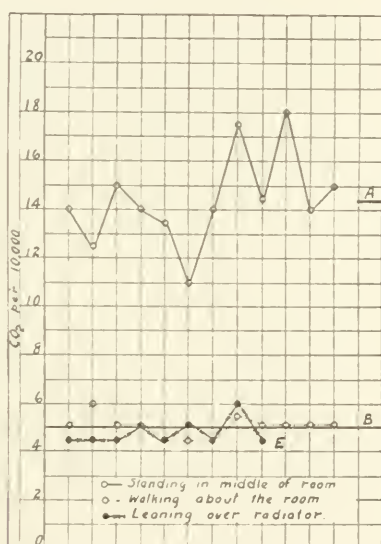


Fig. 10.—(Exp. 10). Showing no reinspiration when walking about the room (*B*), or when leaning over a radiator (*E*), contrasted with 2.1 per cent. when standing still (*A*).

The same result may be obtained by walking about the room, thus creating one's own current of some 200 to 300 feet per minute, and leaving behind at each step the expired breath. The walking may be confined to a very small circle without any change in the results. It is equally effective to stand with the head above a radiator, which, when well heated, will cause an upward convection current of some 200 feet per minute. Figure 10 illustrates these statements very well, and shows that 2.1 per cent. of the expired air was rebreathed when standing still. This experiment was made in the same room as the foregoing, with closed windows and a temperature of 63 F.



Results confirming those above noted are shown in Figure 11. When this experiment was made, the room was receiving 28,000 cubic feet of air per hour from an open window and the temperature was 66 F. On account of the large inflow of cool air, the air near the floor contained only 4 CO<sub>2</sub> per 10,000, while it had 4.5 at a height of 5 feet above the floor. The same difference is detected in curves *B* and *C*, the former of which represents samples of the inspired air taken in the upward current over the radiator, and the latter those taken while walking about the room. When standing quietly in the middle of the room the reInspiration was 1.2 per cent., as shown in the curve *A*.

In connection with the avoidance of reInspiration by induced air currents, the interesting observation has been made that when the back

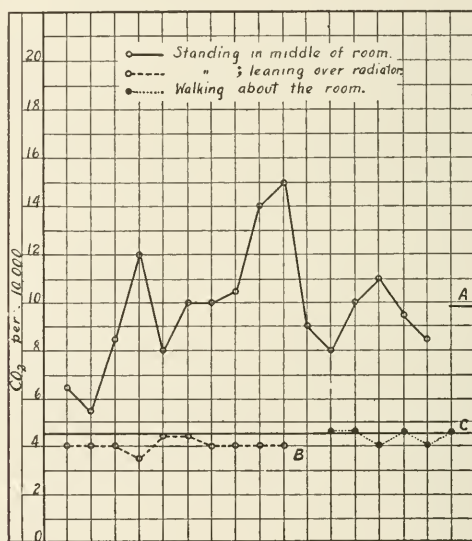


Fig. 11.—(Exp. 6). Showing no reInspiration when walking about the room (*C*), or when leaning over a radiator (*B*), contrasted with 1.2 per cent. when standing still (*A*). The room was receiving 28,000 cubic feet of air per hour from an open window.

is turned to the breeze a little of the expired air is often reinhaled in spite of the current. This is interpreted to mean that the eddies formed in front of the face prevent an immediate removal of the expired air. Two experiments made while standing in a narrow hallway with a very regular flow of air, and which illustrate this, are shown in Figure 12. In that shown at the left the current was just perceptible, presumably about 150 feet per minute; in that at the right the current was 300 feet per minute. The samples in each series were taken alternately, one while facing toward, then one while facing away from, the air current.

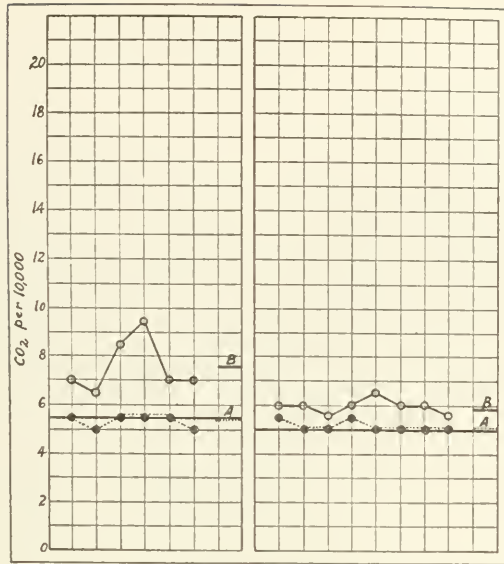


Fig. 12.—(Exps. 3 and 19). Showing that air currents of small velocity do not entirely prevent reInspiration when directed from behind (B), but do when directed from the front (A).

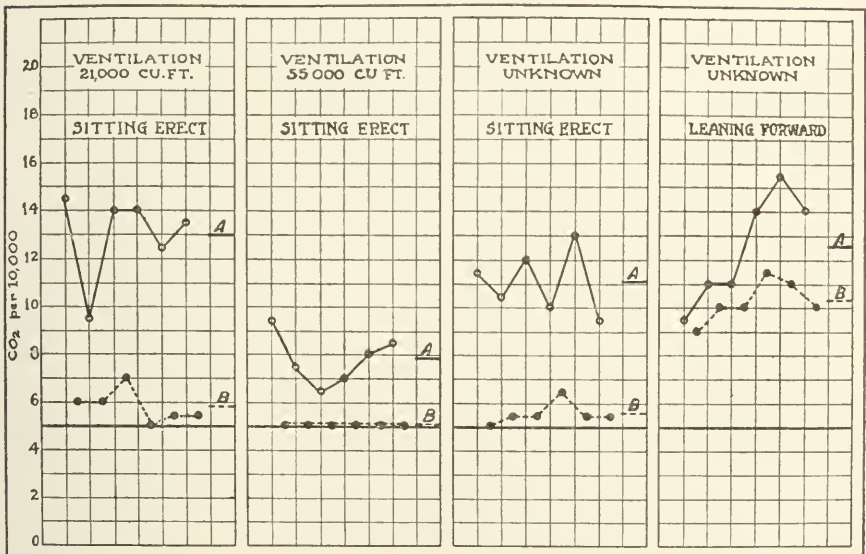


Fig. 13.—(Exper. 2). Showing that there is practically no reInspiration with mouth breathing unless the expired current is directed downward, as in B at the extreme right. A, nasal breathing; B, mouth breathing.

## THE EFFECT OF MOUTH BREATHING

If one sits erect and breathes through the open mouth the expired air is thrown directly forward; only the tip of the cone-shaped expansion lies next to the face, and a very slight motion will carry it out of the zone from which the inspired air is taken. Even the slow convection currents are usually sufficient to cause this slight motion within the necessary time limits. This results in there being practically no re-inspiration with mouth breathing, unless there is such a change of position of the head as to throw the expired current downward, or unless some obstruction is brought in the way of its forward motion. The facts are well illustrated in Figure 13. This experiment was made in a room of 3,000 cubic feet with a temperature of 70 F. The ventilating air came

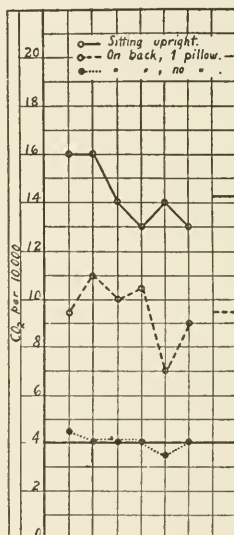


Fig. 14.—(Exp. 16). Showing the effect of position on re-inspiration.

from a hallway and had constantly 5  $\text{CO}_2$  per 10,000. Nasal breathing is represented by the curves marked *A*, and mouth breathing by those marked *B*. The position of the body in the fourth group was that which is usually assumed when seated for writing at a table. The mouth and nasal samples were taken alternately in each of the four groups. Abundant confirmation of these results were noted in other experiments.

## THE EFFECT OF POSITION

Every change of position of the head affects the line in which the expired air is directed. In the normal sitting or standing position (with nasal breathing), as has been previously stated, it is thrown downward, close to the body, and there are more or less constant upward

convection currents which tend to bring it back to the face. If the head is thrown well back the air is thrown away from the body, somewhat as it is in breathing through the open mouth, and re-inspiration is lessened or prevented entirely.

The effect of such changes can be best studied by assuming the recumbent position and lowering or raising the head. Figure 14 shows three short series of observations made under these conditions in a bedroom of 1,500 cubic feet with a temperature of 64° F. While sitting on the bed, propped upright against the headboard, the re-inspiration was

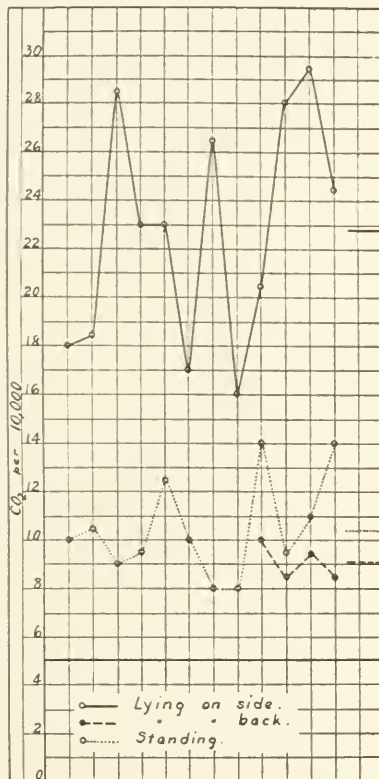


Fig. 15.—(Exp. 5). Showing the effect of position on re-inspiration.

2.3 per cent.; lying down with a pillow tilting the head forward it was 1.3 per cent.; when the pillow was removed and the head thrown far back there was none at all.

It is well known that gases tend to adhere to surfaces with which they come in contact. When one lies with the side of the face on a pillow, the expired air is directed against its surface, and this adhesion will delay its removal from the immediate neighborhood of the face. Furthermore, there is the formation of pockets and dead spaces in the angles which are



not easily acted on by convection currents. The softer the pillow, and the more one's head sinks into it, the greater will be the retention. The result of lying in the position indicated is that relatively large amounts of the expired air are reinhaled. This is illustrated by the experiment shown in Figure 15, made in the same room as the preceding, with a temperature of 60 F. While standing by the edge of the bed the reinspection was 1.2 per cent.; lying with the side of the face on the pillow it was 4 per cent.; lying on the back with a pillow under the head it was 1 per cent. In this, as in the preceding and in all other similar experiments, there

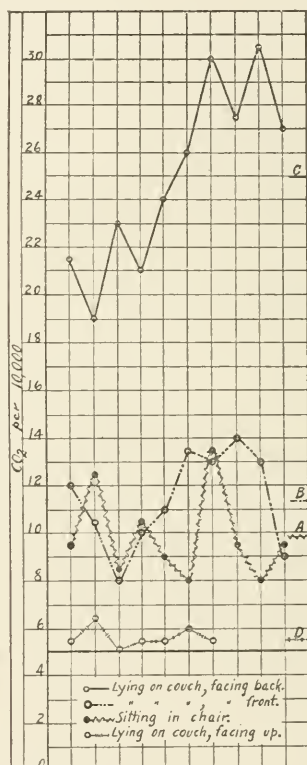


Fig. 16.—(Exp. 11). Showing the effect of position on reinspection.

was no obstruction by blankets or other covering. The only effect of such obstructions would be considerably to increase the amount of air re-inspired.

The effects of position are again illustrated by the experiment shown in Figure 16, which was carried out in a large and well-ventilated sitting room, with an open fire-place and a vigorous fire. The temperature rose while the tests were being made from 64 F. to 72 F. When sitting in a chair some 8 or 10 feet from the fire the reinspection was 1.1 per cent.

(A) ; lying on a couch, with the face toward the edge and a little removed from it, the reinspiration was 1.4 per cent. (B) ; with the face toward the high back of the couch, and some 8 or 10 inches from it, there was 4.4 per cent. reinspiration (C) ; while lying on the back, with a very miniature pillow, there was practically none (D).

As might be readily inferred from the results of the foregoing experiments, it was found that ventilating currents have less effect on reinspiration in the lateral recumbent position than when the head is free. The adhesion of the expired air and the mechanical obstruction about the face

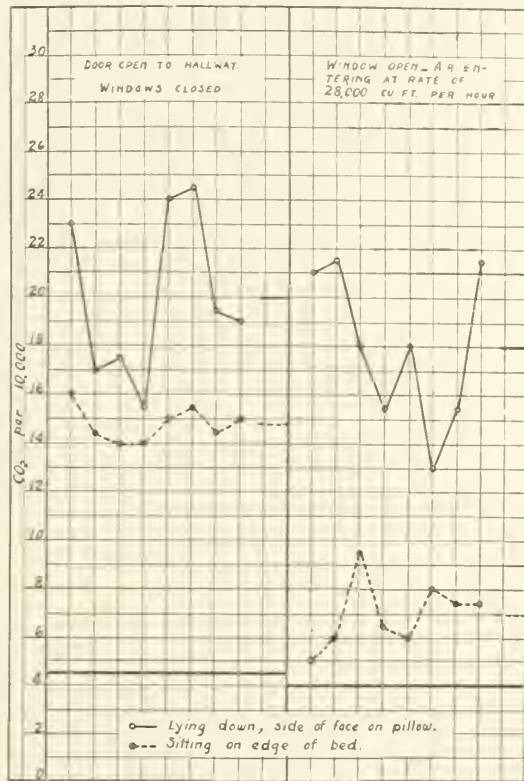


Fig. 17.—(Exp. 7). Showing effect of position and changing ventilation on reinspiration.

prevent the ready action of such currents in bringing about displacement. The condition is illustrated by the experiment shown in Figure 17. This was made in a bedroom of about 1,500 cubic feet, where the temperature was 50 F. With closed windows the reinspiration was 2.3 per cent. and 3.4 per cent. for the upright and recumbent positions, respectively: with an open window admitting air at the rate of 28,000 cubic feet per hour they were 0.7 and 3.1 per cent., respectively.

The difficulty of dislodging the expired air even by artificially produced direct currents, when one breathes over the surface of a pillow, is further illustrated in Figure 18. This experiment was made while lying on the side as in the preceding. The temperature was 70 F. The current of air was from an electric fan placed beyond the feet and nearly in line with the body, but a little behind. It was about 12 feet from the head and 2 feet above the bed. The current was measured just above the pillow—practically in the position assumed by the head when lying there. When the air was not disturbed the reinspiration was 3.6 per

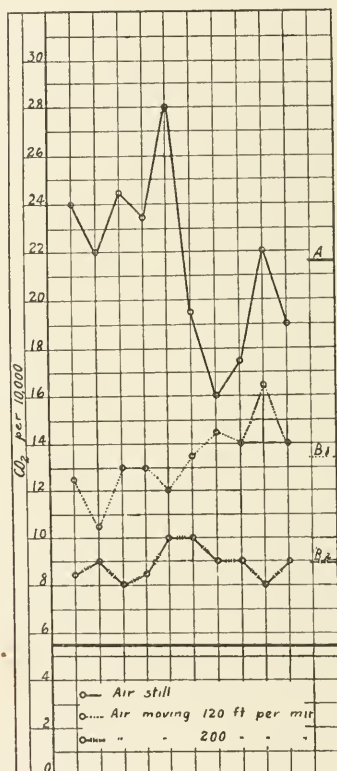


Fig. 18.—(Exp. 13). Showing the effect of air currents on reinspiration while lying with the side of the face on a pillow.

cent. (*A*); with a current of 120 feet per minute it was 1.8 per cent. (*B*<sub>1</sub>), and with a current of 200 feet per minute it was 0.7 per cent. (*B*<sub>2</sub>). With the head free and facing the breeze either of these currents is sufficient to prevent reinspiration entirely.

An air current strong enough to be felt as a breeze may still fail to remove the expired air under the above conditions, even when it flows directly at the face. This is illustrated by Figure 19. The observations

were made in the same place as the above. The temperature was 60 F. When the air was not artificially disturbed there was 2.8 per cent. reinspiration (*A*); with a fan current of 150 feet directed from behind the body there was 2.1 per cent. (*B*); and with the same current directed from in front there was still 1.1 per cent. (*C*). The breeze was quite perceptible on the face.

#### THE EFFECT OF INCREASING AND DECREASING $\text{CO}_2$

The degree of contamination in the surrounding air has practically no effect on the amount of immediate reinspiration. Any increase or decrease of the  $\text{CO}_2$  in the general air of a room is accompanied by corresponding changes in the  $\text{CO}_2$  of the inspired air. The gap between

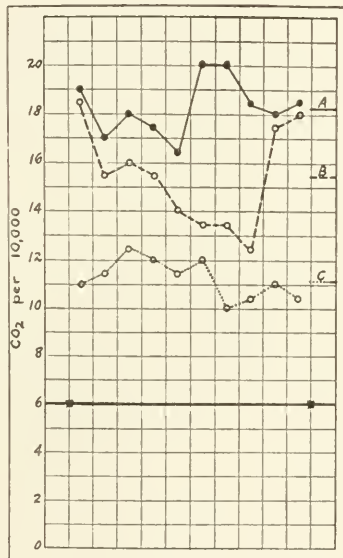


Fig. 19.—(Exp. 26). Showing the effect of air currents from different directions on reinspiration while lying on side. Air still at *A*; a current of 150 feet per minute from behind at *B*, and from in front at *C*.

the two remains about the same wherever they lie in the scale. It is understood, of course, that these statements apply only when other conditions, such as temperature and air currents, are unaltered. After Lehmann<sup>1</sup> had highly contaminated the air of his laboratory by burning gas, he found about the same difference in  $\text{CO}_2$  content between this air and the inspired air that was found on other occasions when the laboratory air was relatively pure.

In order to test the matter in another way, I shut myself in an otherwise empty clothes closet of 175 cubic feet, which could be rapidly contaminated by my own expiration, and made simultaneous observations



of the air of the room and my inspired air at five-minute intervals. After ten samples of each had been collected the door was opened and the place thoroughly fanned for ten minutes to renew the air; then six more samples of each were taken at similar intervals, the door being open, the room vacated, and the air agitated during the intervals in order to prevent any reaccumulation of  $\text{CO}_2$ , but closed while the samples were being taken. The temperature was 78 to 80 F.

During the first period of the experiment the  $\text{CO}_2$  of the air of the room rose in a very regular curve from 6.5 to 16.5 per 10,000, and the

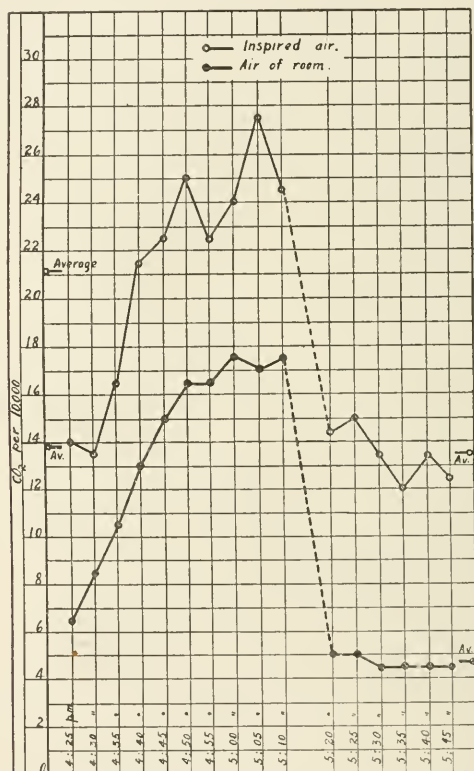


Fig. 20.—(Exp. 15). Showing that changing the  $\text{CO}_2$  in the surrounding air has practically no effect on the amount of reinspiration.

$\text{CO}_2$  of the inspired air rose in an irregular but corresponding curve a like amount. During the fanning-out process the  $\text{CO}_2$  of the air of the room fell to 5 per 10,000, near which it remained during the subsequent tests. The  $\text{CO}_2$  of the inspired air also dropped back to about the starting point and remained there. These results are shown in Figure 20. During the first period the average reinspiration was 1.6 per cent.; during the second period it was 1.9 per cent.

The results of the first part of this experiment were verified on another occasion under similar general conditions, and in addition to the above, a sample of the air inhaled through the open mouth was taken at each five-minute period. The  $\text{CO}_2$  of the air of the room rose from 5 to 14 per 10,000, and averaged 11 per 10,000. The  $\text{CO}_2$  of the inspired air with nasal breathing likewise rose in an irregular curve and averaged 18.3 per 10,000, the difference (7.3 per 10,000) being equivalent to a reinspiration of 1.6 per cent. The  $\text{CO}_2$  of the inspired air with mouth breathing followed very closely that of the air of the room; they averaged within a small fraction of each other, thus showing practically no reinspiration while breathing through the open mouth, as has been previously stated.

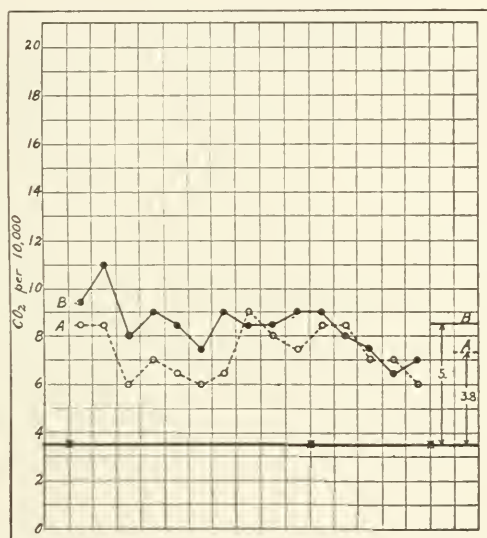


Fig. 21.—(Exp. 28). Showing reinspiration in a sleeping-porch when sitting near the center (A), and while lying on a bed near an open side (B).

#### THE SLEEPING-PORCH AND THE OPEN AIR

From the experiments recorded it is evident that very slight interference with the free movement of air currents about the face is sufficient to prevent the immediate removal of the expired air and to allow contamination of the air we breathe. It was believed that such interference might easily occur in the ordinary sleeping-porch, which is usually built with two open sides, or, sometimes, even in the open air. Investigation of the inspired air under these circumstances was made, and the results found to be in harmony with those previously obtained. One does not necessarily breathe pure air because he is out of doors; he is not at all likely to do so under the ordinary conditions of sleeping in tents, tent-houses, or half-open porches, such as are used for therapeutic or hygienic purposes.

An experiment carried out in a sleeping-porch 15x13x8.5 feet is shown in Figure 21. The porch has open sides to the south and east, covered by wire screens of about 12 meshes to the inch. The temperature was 70 F., and there was a breeze of 400 to 500 feet per minute in the outside air, varying in direction between south and southwest. The air of the porch, and outside of it, contained 3.5 CO<sub>2</sub> per 10,000, as shown by three successive determinations made in duplicate and indicated on the base line in the chart. This porch contained two beds, one of which stood near the center and opposite a door in the north wall, and one of which was close to the open east side. Two series of tests were made: one while sitting on the edge of the center bed (A), and one while lying on the other bed with the face about 2 feet from the screen (B). The re-inspiration was 0.8 per cent., and 1.1 per cent. for the two series, respectively.

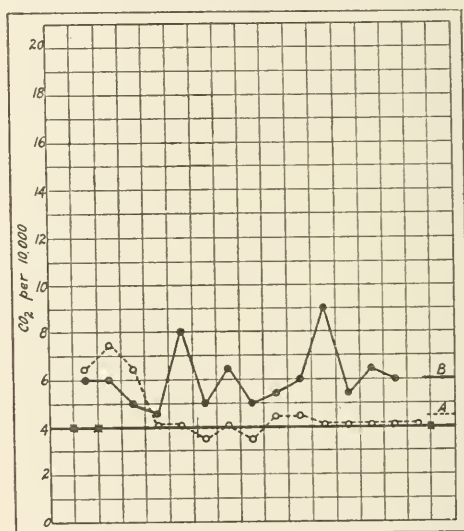


Fig. 22.—(Exp. 29). \*Showing the inspired air when out of doors. A, standing free; B, on an open porch.

Lehmann<sup>1</sup> made four observations in the outside air and found the difference between the CO<sub>2</sub> of the inspired air and of the surrounding air to be 0.6, 0.3, 0.8 and 0.0 per 10,000. The difference is so small as to indicate practically no re-inspiration. I have made similar findings. The outside air is rarely still, and it moves in great masses. If there is no obstruction the expired air is quickly removed from the breathing zone. If one stands perfectly free there will be no re-inspiration, except occasionally on very still days. But it is only necessary to step behind an obstruction of some sort to break up the progressive mass movement of the atmosphere, and the phenomenon begins again. These two conditions are shown in Figure 22. There was a light breeze blowing and the temperature was 70 F. The curve A shows a series of tests while

standing well away from any obstruction. Another person was standing only a few feet distant and on the windward side. It is possible that his expired air accounts for the excess of  $\text{CO}_2$  in the first three samples of this series. The curve *B* shows a series while seated on an open porch built against the side of the house and opposite to the direction of the wind. The reinspiration is not great, but it is present. The inspired air is not pure, and the experiment serves to show how necessary it is that the air currents be free and unobstructed in order that uncontaminated air may enter the nostrils.

The experiments illustrated and referred to in the preceding represent only such conditions as are likely to be met with in daily life. With the single exception of the closet, the rooms utilized were well ventilated. Many times the air-supply was excessive. The attempt has been made to determine what kind of air is breathed under ordinary and good conditions, rather than what may be breathed under exceptionally bad conditions. It may be fairly concluded that when one lives indoors and remains quiet he will immediately rebreathe from 1 to 2 per cent. of his own expired air. When he goes to bed it will be more — from 1 or 2 per cent. to 4 or 5 per cent., depending on the position in which he lies. In some of the positions assumed by people sleeping it may even be as high as 8 or 10 per cent.; and I have once found it 18 per cent. in a single test, which did not necessitate a position by any means improbable. Nor does sleeping in the open insure pure air for breathing. The same influences here produce the same relative results that they do inside. When one buries his head between pillow and bed-clothes for the sake of warmth, reinspiration is inevitable, and it is not necessarily small in amount.

#### THE SIGNIFICANCE OF REINSPIRATION

The phenomenon of reinspiration has been recorded with rather less detail than was observed in the experiments, but with enough to show that it must be a very common occurrence. In order to understand its significance it is necessary to consider certain phases of the physiology of respiration.

Haldane and Priestly<sup>4</sup> have shown that the regulation of breathing is dependent on the concentration of carbon dioxide in the air-cells of the lungs. By the proportion of  $\text{CO}_2$  in the air-cells the concentration of this gas in the arterial blood is determined, and the nerve cells controlling the respiratory movements are stimulated by the carbonic acid in the blood supplied to them. Hough<sup>5</sup> confirmed these findings. He concludes, from

4. Haldane and Priestly: The Regulation of the Lung Ventilation, *Jour. Physiol.*, 1905, xxxii, 225.

5. Hough: Variations in the Response of Healthy Men to the Dyspneic Conditions Produced by Breathing a Confined Volume of Air, *Am. Jour. Physiol.*, 1911, xxviii, 369.



experimental evidence, that "the increase of pulmonary ventilation is entirely the result of increase of the  $\text{CO}_2$  tension in the blood flowing through the respiratory center"; and Henderson<sup>6</sup> believes that "the *capnicity* of the body [the concentration of  $\text{CO}_2$  in the blood] is automatically maintained with a precision equalling that exhibited in the regulation of body temperature."

The carbon dioxid which is being constantly formed in the body is carried to the lungs by the venous blood. It escapes from the blood into the air-cells of the lungs, and its escape is impeded or accelerated according to the resistance it meets in them. This resistance depends on the proportion of  $\text{CO}_2$  in the alveolar air, since the tension of this gas in the blood can fall only as low as it is on the other side of the membrane separating the blood-stream from the air-cell. The arterial blood leaves the lungs with essentially the same pressure of  $\text{CO}_2$  that is found in the alveolar air. It is in this way that the alveolar  $\text{CO}_2$  regulates the  $\text{CO}_2$  tension in the blood, and so controls the respiratory movements.

Each person's breathing is so regulated as to maintain the percentage of  $\text{CO}_2$  in the alveolar air at about 5 per cent. of an atmosphere. If the pressure falls below this, respiration is lessened or stopped until the loss is regained; if it goes above 5 per cent., the respiration is increased until the normal is restored. With light muscular work the  $\text{CO}_2$  formed in the body will be doubled or trebled, and the breathing will be increased in like proportion so as to remove the  $\text{CO}_2$  from the air-cells just fast enough to maintain the alveolar tension at the normal 5 per cent., but not lower. Douglas and Haldane<sup>7</sup> found that from lying in bed to walking five miles per hour the  $\text{CO}_2$  output was increased twelve times, and that the alveolar ventilation was likewise increased twelve times, so that the percentage of  $\text{CO}_2$  in the alveolar air remained practically constant. Henderson<sup>6</sup> found no material change in the composition of the alveolar air on going from rest to strenuous exercise; the increased production of  $\text{CO}_2$  was perfectly compensated for by increased breathing.

Haldane and Priestly<sup>4</sup> found not only that the percentage of  $\text{CO}_2$  in the alveolar air remains constant under all degrees of exertion, but that its absolute partial pressure is unaltered when the general pressure is reduced to two-thirds of an atmosphere. That the same rule holds good in the increased pressure of caissons having up to 6 atmospheres has been shown by Hill.<sup>8</sup> In his experiments he found the alveolar air to contain 5.4 per cent. of  $\text{CO}_2$  at 1 atmosphere, and it gradually fell, with increasing pressure, to 0.9 per cent. at 6 atmospheres, the percentage

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6. Henderson and Russell: A Simple Method for Determining the Carbon Dioxid Content of the Alveolar Air, *Am. Jour. Physiol.*, 1912, xxix, 436.

7. Douglas and Haldane: The Dead Space of the Respiratory Passages, *Jour. Physiol.*, 1912, Abst. in *Brit. Med. Jour.*, Nov. 16, 1912, p. 1411.

8. Hill, Leonard: Caisson Sickness. Edward Arnold, London, 1912, p. 47.

changing in an inverse ratio to the increase of external pressure, so that the absolute tension of  $\text{CO}_2$  in the alveolar air always remained at about 5 per cent. of the pressure of one atmosphere.

Haldane and Poulton<sup>9</sup> have shown that if the  $\text{CO}_2$  is first removed from the lungs by rapid breathing, apnea may coexist with actual want of oxygen, and that breathing does not begin again until the alveolar  $\text{CO}_2$  has returned to the normal.<sup>10</sup> Alveolar air normally contains about 16 per cent. of oxygen, and the red blood-cells leave the lungs practically saturated with it. The amount taken up on their next trip through the lungs depends on how much they have given up to the tissues in the meantime, not on how much is available to their use. The normal 16 per cent. of oxygen in the alveolar air is automatically maintained by the action of the  $\text{CO}_2$  on the respiratory center, but on account of the chemical affinity of the hemoglobin for oxygen the blood-cells may still take practically their full capacity when it is reduced to 12 per cent. or less in the alveolar air. Thus a large excess of oxygen is constantly maintained in the air of the lungs. And while it is one of the chief functions of respiration to supply oxygen to the body, neither a surplus nor a deficiency of it, unless the alteration is extreme, have any effect on the respiratory movements. Breathing will not be lessened nor more oxygen taken up because more of it is supplied to the lungs; nor will the oxidation processes in the body be affected in any way, unless other influences are simultaneously brought into play.

With each breath we take back into the lungs the air contained in the nose and larger bronchi — the “dead-space” air. This dead-space air constitutes about one-third of the whole volume of quiet inspiration, and not less than one-tenth of deep breathing. To all intents and purposes it is expired air which is constantly re-inspired. The phenomenon of re-inspiration now under discussion is in effect simply a slight extension of the dead-space beyond its necessary limits. Rebreathing of the ordinary dead-space air is a normal and conservative process; it prevents pure air from entering the lungs and reducing the  $\text{CO}_2$  below the amount required for stimulating the respiratory center; it makes of breathing a regular and continuous rather than an irregular and interrupted function. Douglas and Haldane<sup>7</sup> have recently shown that the volume of the dead-space, instead of being a fixed quantity, is automatically altered so as to give greater or less resistance to the air-flow to and from the lungs with changing exertion. They go so far as to state that rather marked variations may occur; and, while the mechanism is not fully understood, they

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9. Haldane and Poulton: The Effect of Want of Oxygen on Respiration. *Jour. of Physiol.*, 1908, xxxvii, 390.

10. Unless oxygen has fallen so low in the body that imperfect oxidation in the processes of metabolism causes the formation of abnormal acids, notably lactic, which reinforce the  $\text{CO}_2$  in the blood for action on the respiratory center.

think the regulation is as perfect as is that of the size of the arterioles for controlling the blood-flow.

There can be no doubt that there is a large measure and a wide range of physiologic response on the part of the respiratory function to meet changing external as well as internal conditions of  $\text{CO}_2$  concentration. It has been seen that when more  $\text{CO}_2$  is formed in the body the respiration is automatically increased in like proportion, and in this way the alveolar  $\text{CO}_2$  is kept at a uniform level of 5 per cent. The same thing happens when we breathe an atmosphere containing an excess of  $\text{CO}_2$ . The volume of air breathed is then increased in such a degree as, if possible, to keep the  $\text{CO}_2$  in the alveolar air normal. Haldane and Priestly\* found that with 2 per cent. of  $\text{CO}_2$  in the inspired air the

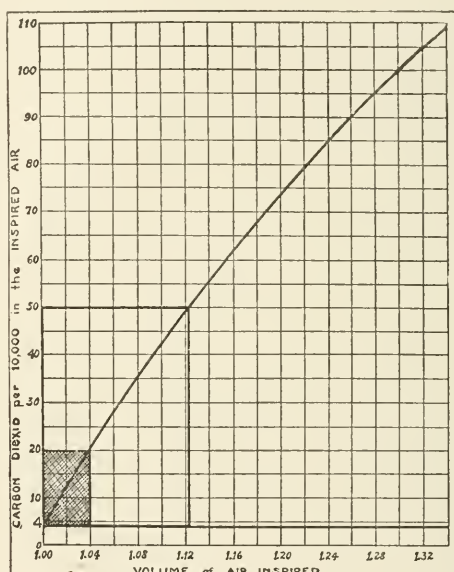


Fig. 23.—Showing the increasing depth of inspiration required to maintain a constant proportion of  $\text{CO}_2$  in the air of the lungs, while the production of this gas in the body remains constant and its proportion in the inspired air increases.

pulmonary ventilation is increased 50 per cent.; with 3 per cent. it is increased about 100 per cent.; with 4 per cent., about 200 per cent.; with 5 per cent., about 300 per cent.; and with 6 per cent., 500 per cent. With the last the alveolar tension of  $\text{CO}_2$  is, of course, above the normal, and this fact is signified by severe panting; but up to 3 per cent. in the inspired air the increase of breathing is scarcely noticed, unless muscular work is done, when the increased internal production of  $\text{CO}_2$  calls for a still greater increase of the pulmonary ventilation. The adjustments are automatic and go on without our consciousness, unless an excessive increase of breathing is demanded.

If we are to maintain a constant proportion of  $\text{CO}_2$  in the alveolar air, each inspiration must be just large enough to dilute to the same degree the  $\text{CO}_2$  produced in the time interval of one respiration. If the amount of  $\text{CO}_2$  produced is constant, each breath will require the same amount of air to maintain the equilibrium. But if the air used for this dilution already contains some  $\text{CO}_2$  more will be required than if it had none; and as the amount of  $\text{CO}_2$  in the inspired air increases, an increasingly larger amount will have to be inhaled in order to maintain the alveolar constant of dilution. Mathematical calculation shows that the volume of pulmonary ventilation required to maintain a constant proportion of  $\text{CO}_2$  in the alveolar air, while the production of this gas by the body remains constant and its proportion in the inspired air increases, takes the form of an asymptotic curve. Figure 23 shows the form and dimensions of this curve of increasing depth of breathing for proportions of  $\text{CO}_2$  in the inspired air ranging from 0.04 to 1.1 per cent.<sup>11</sup>

The normal air contains not more than 0.04 per cent. of  $\text{CO}_2$ . In well-ventilated rooms it is 0.06 or 0.08 per cent.; in poorly-ventilated rooms it may be 0.2 per cent. or more, but under very bad conditions it will rarely exceed 0.5 per cent. Including the ordinary amount of immediate reinspiration, the  $\text{CO}_2$  of the inspired air is not liable to exceed 0.5 per cent., and it will practically never go beyond 1.0 per cent. The small shaded quadrangle at the left lower corner of the chart, therefore, includes that portion of the curve corresponding to general contamination of the air in poorly-ventilated rooms, while the heavy outline beyond this includes the portion corresponding to this contamination plus the contamination due to immediate reinspiration. Four per cent. and a fraction over 12 per cent. are seen to be the respective limits of respiratory increase required to maintain the alveolar  $\text{CO}_2$  at a constant proportion, while 1.0 per cent. of  $\text{CO}_2$  in the inspired air requires only 30 per cent. increase of the depth of breathing to maintain the same constant. When we remember that the possibility of increase in the depth of inspiration is 400 or 500 per cent., and that by changing the

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11. The unit volume of the tidal air when the inspired air is pure (4  $\text{CO}_2$  per 10,000), is assumed to be one-fifth of the volume remaining in the lungs at the end of expiration. This is about the average for quiet breathing. Hough<sup>5</sup> and Douglas and Haldane<sup>7</sup> have shown that increase in the pulmonary ventilation is brought about chiefly by increasing the depth of inspiration, supplemented when necessary by an increase in the rate of breathing and then by increased expiratory effort. The curve in the chart is constructed to depth of inspiration only, and assumes not only a constant rate of production of  $\text{CO}_2$  in the body, but a constant rate of breathing and a constant volume of air in the lungs at the end of expiration. The figures in the chart are essentially correct for the increasing minute-volume of pulmonary ventilation, whatever the change in breathing by which this is brought about, when the unit is based on the removal by the outgoing tidal air of an average production of  $\text{CO}_2$  by the resting body, which is really the better way to express the relation.



rate and the completeness of expiration the alveolar ventilation may be increased considerably more than 1,000 per cent., it is readily understood why this slight adjustment of 4 to 12 or even to 30 per cent. falls far below the limits of our conscious effort. It has already been stated, from the experiments of Haldane and Priestly<sup>4</sup> that an actual increase of 100 per cent. in the pulmonary ventilation passes almost unnoticed.

In other articles I have reviewed<sup>12</sup> the evidence concerning volatile poisons in the expired air, a subject which has always occupied a prominent place in discussions of the air we breathe. To one who had followed the large amount of experimental investigation by many workers it seemed that the question of their existence had been finally answered in the negative in 1905, when Flügge<sup>13</sup> and his associates demonstrated clearly that the ill effects of confined air need no such hypothetical poison for their adequate explanation. It seemed for a time as if Weichardt,<sup>14</sup> by the application of some new methods of investigation, had reopened the question; but Inabe<sup>15</sup> and Amoss,<sup>16</sup> in attempting to confirm his work, have pointed out such faults of technic as practically to reverse his results and negative his conclusions. Moreover, the absence of these volatile poisons has been recently maintained by Hill,<sup>17</sup> who has added much new evidence and a large measure of support for the contentions of Flügge.

On the other hand, the recent experiments of Rosenau and Amoss<sup>18</sup> indicate that the condensed water vapour of the human breath may contain a trace of organic matter belonging to the protein group. When this fluid was injected into animals about one-fourth of them were so sensitized that a subsequent injection of human blood-serum gave a distinct anaphylactic reaction. This work will undoubtedly be repeated and confirmed, or another explanation offered. No volatile proteins have been previously known, none have been otherwise demonstrated in the breath, and the condensed water itself has no toxic effect when injected into animals. And when we remember that so small a quantity of egg albumin as 1-20,000,000th of a milligram<sup>19</sup> is sufficient to confer a similar

12. Crowder. *THE ARCHIVES INT. MED.*, 1911, vii, 85; *Trans. of the Section on Preventive Medicine and Public Health, Am. Med. Assn.*, 1911, 177; *THE ARCHIVES INT. MED.*, 1913, xi, 66.

13. Flügge, Heymann, Paul, Erelenz: *Ztschr. f. Hyg.*, 1905, xlix, 363, 388, 405, 433.

14. Weichardt: *Arch. f. Hyg.*, 1908, lxxv, 252; 1911, lxxiv, 185.

15. Inabe: Ueber das Kenotoxin Weichardts in dem Ausathmungsluft, *Ztschr. f. Hyg.*, 1911, lxxviii, i.

16. Amoss: Organic Matter in the Expired Breath, *Jour. of Exper. Med.*, 1913, xvii, 132.

17. Hill, Leonard: The Influence of Ozone in Ventilation. *Jour. Royal Soc. Arts*, 1912, lx, 344.

18. Rosenau and Amoss: Organic Matter in the Expired Breath, *Jour. Med. Research*, 1911, xxv, 35.

19. Wells: Studies of the Chemistry of Anaphylaxis, *Jour. Infect. Dis.*, 1908, v, 449.

sensitization, it would seem that there may be possible sources of error quite difficult to control entirely.

But leaving all this aside, suppose for the moment that the breath does contain a protein — or any other waste organic substance — it still does not appear that a little reexpiration could be harmful. In the alveolar air this substance would necessarily be present in relatively high concentration, but always a lower concentration than the blood or tissues from which it comes. At the end of expiration it would fill the dead-space in like concentration, as must any gas excreted into the lungs, and the dead-space air is all drawn back with each breath as a part of normal breathing. We cannot directly increase the concentration of this hypothetical substance in the blood by breathing an atmosphere containing a lower concentration of it; we can only hinder its escape. But in this there is a necessary relation between any such hypothetical body in the expired air and the  $\text{CO}_2$ , as well as a striking analogy to  $\text{CO}_2$  as a poison which has been clearly drawn by Sewall.<sup>20</sup> We cannot rebreathe expired air without rebreathing  $\text{CO}_2$ , and rebreathing  $\text{CO}_2$  causes deeper inspiration. The alveolar concentration of any gaseous constituent of the expired air must therefore be subject to the same laws of compensatory change in the pulmonary ventilation as are determined by the  $\text{CO}_2$ . Our hypothetical substance would be regulated to whatever may be at any time its normal proportion simultaneously with, and by the action of, the  $\text{CO}_2$ . And the quantitative values of the curve presented in Figure 23 will apply exactly to any other volatile excretion into the air-cells by substituting for  $\text{CO}_2$  the percentage of expired air represented by the  $\text{CO}_2$ . Let us assume that it were possible, by higher concentrations of our hypothetical organic body, to poison the animals producing it; it is possible also to poison them by  $\text{CO}_2$  — or even by oxygen — but far from being harmful in its normal concentration  $\text{CO}_2$  is a prime necessity; and the remarkable adaptability of the respiratory function enables the body to maintain a normal concentration under a great variety of conditions. Regulation is automatic, and, according to our present physiologic conceptions, necessarily takes place. The adjustment demanded by the ordinary amount of reexpiration is relatively very small, and it would seem that it must lie well within the margin of safety which Melzer<sup>21</sup> has so well described as belonging to all well-understood physiologic processes.

It is commonly, though erroneously, supposed that the good effects of efficient ventilation are due to the chemical purity of the air. When attention was called to the occurrence of immediate reexpiration it was looked on as a newly discovered source of impurity in the air we breathe.

20. Sewall: On What Do the Hygienic and Therapeutic Virtues of the Open Air Depend, *Jour. Am. Med. Assn.*, 1912, lviii, 174.

21. Melzer: Factors of Safety in Animal Structure and Animal Economy, *Harvey Lectures*, New York, 1907-8, p. 139.

To this previously unrecognized source of contamination was attributed much of the failure that has so often attended attempts to bring comfort out of ventilating procedures, and from this has arisen the "theory of displacement" in its application to ventilation. Accepting the old notion that chemical purity is a proper basis for ventilation standards, and assuming that reinspiration is necessarily harmful, some of the hygienists propose to handle the air supplied to a room in such a manner that the expired air will be immediately carried away from the face and cannot be rebreathed. It is asserted that if the new principle is applied a much smaller quantity of air than is demanded by the older quantitative or dilution standards can be made to yield hygienic results. But small quantities of air do not lend themselves readily to maintaining the currents that have been shown to be necessary in order to accomplish the displacement aimed at. Or, if the necessary currents are maintained, and throughout a sufficient area to be effective, it becomes a physical necessity to recirculate the air; thus only delaying rather than preventing reinspiration, and reducing the system to one of simple dilution, which it is the avowed purpose to avoid. By actual experiment I know that the plan most widely heralded does just this thing so far as the contamination of the air of a room is concerned, and it is improbable that immediate reinspiration is materially restricted. The theory of displacement does not sufficiently take into consideration that all animals possessing lungs ventilate them on a very simple principle of dilution; nor does the pure air theory sufficiently consider that the air of the lungs always remains highly contaminated with their own excretory gases, and that there is such an effective barrier as the dead-space against the lowering of the contamination.

If it is desired to prevent reinspiration, ways have been indicated by which this may be done. The results will be good, bad, or indifferent, according to the plan chosen; but in no case will the results, whether good or bad, depend on the fact that reinspiration is prevented. Within a wide range of variation in the purity of the air we breathe (in so far as the purity is effected by the products of respiration), the respiratory function is perfectly adapted to maintain its normal balance. The only apparent effect of rebreathing a little of our own expired air is a slightly deeper inspiration.

But ventilation is not a matter of little consequence because of this. It is still just as important as it has always been considered; the benefits of fresh air and the outdoor life are beyond question. But these measures should be carried out in the interest of the heat economy of the body rather than with regard to the chemical purity of the air we breathe. The good effects of the outdoor air depend on its coolness, its motion and its relative humidity. These physical qualities enable it to absorb the

heat which is constantly being formed in the body, and which must be as constantly removed. Air that will take it up rapidly will stimulate healthy functions; air that takes it too slowly leads to sluggish metabolism, and if maintained will ultimately result in a low resistance to disease.

The rigor of a temperate or a colder climate makes of its inhabitants a house-dwelling race. They very commonly over-heat their houses, if not by fire and steam then by the heat of their own bodies; and when they do this they complain of poor ventilation, regardless of whether the air-supply is large or small. Whichever this may be, under any conditions that are likely to arise, there will still be oxygen in excess of every demand, and the  $\text{CO}_2$  will still find a ready escape from the blood; but in an over-warm atmosphere the body heat will be stagnated and the consumption of oxygen by the tissue cells will be decreased. Ventilation is necessary in order to maintain the thermic balance of the body and to stimulate its chemical activity; and ventilation with cool air is especially desirable. A little extension of the dead-space beyond the tip of the nose is of no consequence. In spite of this extra contamination of the inspired air, the proportion of  $\text{CO}_2$  in the alveolar air will remain a little lower in a warm room than in the invigorating cold of the out-of-doors, as has been shown by Boycott and Haldane.<sup>22</sup> It will remain so because metabolism is reflexly retarded by a warm aerial envelope, the consumption of oxygen by the tissues and the production of  $\text{CO}_2$  by them being much less in warm air than in cold.

That this discussion concerning the significance of reInspiration applies only to healthy persons scarcely needs to be added. The capacity of the lungs may become so restricted by disease that the slightest addition to their work is undesirable. But when we learn that they are still capable of performing the respiratory function with the capacity reduced to so little as one-sixth of the normal,<sup>23</sup> the margin of safety is seen to be a very generous one.

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22. Boycott and Haldane: The Effects of Low Atmospheric Pressure on Respiration. *Jour. Physiol.*, 1908, xxxvii, 335.

23. Bernard et Mantoux: Capacité pulmonaire minima compatible avec la vie, *Jour. de physiol. exper.*, 1913, xv, 16; (Ed. Abstr. in *Jour. Am. Med. Assn.*, 1913, lx, 1794).



## FUNCTIONAL TESTS OF THE KIDNEY IN UREMIA \*

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In any disease in which the diagnosis cannot be reduced to something like certainty, the prognosis must evidently be considered with some reservation. If in addition diagnosis and prognosis are alike made dependent on laboratory tests, either these procedures must be of demonstrated accuracy and especial relevance or else we shall be led into blunders in diagnosis or faulty deductions in our conceptions of the nature of the disorder.

In relation to uremia, I wish to call attention to certain limitations of laboratory aids, and to exceptions which many cases present to what is perhaps a doubtful average, since by constancy of iteration some of these tests bid fair to be accepted as criteria.

Now, in the conditions we call uremia the clinical manifestations cover a wide range, and in proportion as the picture is varied and changeable the difficulties of correct interpretation are increased. It is just because there is no definite symptom-complex, no fixed association of physical signs correlated with uremia, that its recognition is often held to be simple, and yet the necropsy fails to confirm the diagnosis. In the absence of a more clearly defined clinical entity during life we must depend ultimately for the criterion on the anatomical changes found by the pathologist after death. The only exception, possibly, should be in those cases that have been under observation for long periods before the advent of uremia and in which this state is the logical culmination of events.

The cause of uremia is not known, but the common conception relates the condition to nephritis; whether by functional failure of the kidneys or otherwise, does not at present matter. At necropsy marked renal degeneration is requisite for confirmation of the diagnosis, and with this usually is found edema of the brain and often colitis. Since it is the conception of uremia that it is intimately related to severe nephritis and represents the *denouement* of a pathological process dependent on renal disease, or of which renal disease is an invariable accompaniment, it follows that the value of a test having for its aim the estimation of renal function, can be measured by the results elicited in demonstrated cases

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\* Submitted for publication July 16, 1913.

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of uremia. If this assertion be not correct the only alternative is that uremia is not necessarily related to renal function.

The functional test for the kidneys that is being most generally employed by both internists and surgeons in America is that of phenolsulphonephthalein. In many cases of uremia the results secured by this test accord with the clinical data secured by other procedures. Cases of uremia often fail to eliminate in two hours enough of the substance to permit of quantitative estimation, and such patients sometimes die, sometimes improve sufficiently to leave the hospital. But there are notable exceptions in the operation of the test to which I wish to call special attention.

I shall cite but three cases representing three different types of nephritis.

CASE 1.—E. P., aged 53, was refused life insurance in 1909 in consequence of which he came to his physician; examination revealed moderate secondary anemia, cardiac hypertrophy without valvular disease, blood-pressure 190, arteriosclerosis and enlarged liver. The urine was increased in amount; nycturia was present. In a twenty-four-hour specimen, 2,300 c.c., there was albumen, 1 + grams per liter, many granular casts and leukocytes. Diagnosis, secondary contracted kidney. In November, 1911, marked albuminuric retinitis developed. During December diarrhea was an obstinate symptom. The phenolsulphonephthalein test resulted in 53 per cent. being recovered in two hours. During January marked improvement took place. Another phenolsulphonephthalein test showed 55 per cent. recovery. There was apparently gradual improvement till the middle of February, when after one day of headache the patient had a severe convulsion; coma supervened and continued till death, thirty hours later. Postmortem: Anatomical diagnosis, chronic nephritis (red granular kidney), cardiac hypertrophy and dilatation, pulmonary edema, cerebral edema.

CASE 2.—C. H., aged 46, was admitted to hospital four days before death complaining of extreme vertigo and cramps in the legs. On admission the patient was drowsy and in twenty-four hours there was coma. Examination was negative except for retinal hemorrhages; heart not hypertrophied; blood-pressure 130; respiration normal in character and rate; incontinence of urine; catheterized specimen contained a trace of albumin and an enormous number of casts. Phenolsulphonephthalein test gave 63 per cent. recovery in two hours. Clinical Diagnosis: Chronic interstitial nephritis, uremia. Anatomical Diagnosis: Chronic nephritis, small granular kidney, cardiac dilatation, ulcerative colitis, edema of brain.

CASE 3.—B. F., aged 33. For a month there had been increasing dyspnea and swelling of abdomen and ankles. Examination showed moderate general anasarea, some fluid in abdomen and both pleural cavities. Heart normal, blood-pressure average, 98 millimeters of mercury. Retinae normal. Urine: Average twenty-four voidings 1,100 c.c. Albumin constantly over 2 per cent.; many granular casts; some red blood-cells; chlorids diminished. Clinical Diagnosis: Parenchymatous nephritis.

The course of the disease appeared favorable, as the edema subsided rapidly, several phenolsulphonephthalein tests were done along with other tests: the last, eleven days before death, showed a recovery of 57 per cent. in two hours. Without prodromal symptoms other than slight nausea this patient had a severe convulsive seizure lasting nearly an hour and died shortly after. Anatomical Diagnosis: Chronic parenchymatous nephritis; large white kidney; cerebral edema; edema of lungs.

These cases represent the types of renal lesion that we most often meet. The diagnosis was made correctly in each instance and was proved by necropsy. The phenolsulphonephthalein test gave a figure approximating normal in all, yet, in view of the termination of each case, one would hardly venture to assert that the renal function was normal. As to prognosis, this test would have indicated that recovery was almost certain. Granting that in many cases, perhaps the majority, the results of this test harmonize with the clinical picture and the course of events, we must assuredly find out in what percentage of cases of renal disease we are apt to be misled before we can venture any opinion whatever as to the clinical value of the test either diagnostically or prognostically. Does not the record of the above-cited cases suggest that the excretion of phenolsulphonephthalein depends on some other factor (circulation?) than pure renal disease, which by its presence or absence determines the rate of secretion by the kidney? Non-protein nitrogen of the blood?

In the last few years there is notable in the literature, especially that of Germany, an ever-increasing insistence on the significance of the non-protein nitrogen (residual, filtrate, incoagulable nitrogen) of the blood. It is to be recalled that this is no new method of studying nephritis, although our technic at present may be better than formerly. Bright's cases were investigated by Babbington. Von Jacksch, Hoppe-Seiler, and Ascoli made many determinations on all sorts of cases and came to various opinions. Here too we have to do with a factor of degree. When the non-protein nitrogen is so large, as in some cases up to 200 milligrams per 100 cubic centimeters of blood, the outcome for the patient is pretty definitely bad. But those figures even in uremia are not the rule, and it must also be definitely stated that it is not at all unusual to find the non-protein nitrogen within the normal variation in cases of chronic interstitial nephritis with high blood-pressure—the very type of case in which the method is supposed to be of greatest clinical significance.

The normal limits for the non-protein nitrogen of the blood are according to various authors between 20 and 40 milligrams per 100 cubic centimeters. For normal controls I have utilized cases in the surgical service such as fractures and hernias, in which there was no evidence of renal or cardiovascular disease. With these individuals the average non-protein nitrogen is 32 to 33 milligrams in 100 cubic centimeters of blood, with 44 milligrams as a maximum.<sup>1</sup> Strauss believes that in parenchymatous nephritis the non-protein nitrogen is usually low, which is probably correct in cases of purely tubular involvement in which the process has not been of long duration, but the non-protein nitrogen is

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1. The average non-protein nitrogen in seventy-two cases of uremia in my series was 87 mg. per cent.

occasionally low in cases of contracted kidney also. Figures under 40 milligrams in 100 cubic centimeters of blood in these cases with uremia are not very exceptional in my series.

One is then confronted not by a positive fact which excludes uremia, but by a negative observation to be interpreted along with other clinical data. No one has gone to such arbitrary extremes as Widal,<sup>2</sup> who, in a recent publication classifies uremia according to retention theories: (1) Those patients who retain NaCl and develop edema (*chlorurémique*). (2) Those who show nitrogen retention; that is to say, have an increase in the non-protein nitrogen of the blood (*azotémique*). (3) A combination of the two above classes.

As to the type *azotémique*, it is set forth that when the non-protein nitrogen is 50 to 100 milligrams in 100 cubic centimeters of blood, the prognosis is not immediately fatal; above 100 milligrams, it is to be estimated in months and weeks. Then what is to be said of the following?

CASE 4.—A. T., aged 54. Slowly failing health for several months; notable in loss of strength; rapid fatigue on exertion and slight dyspnea; no history of edema. Three days before admission to hospital, severe vertigo. For two days there was some headache and the patient felt very drowsy.

Examination showed retinal hemorrhages, very slight cardiac hypertrophy, blood-pressure 140 to 150 millimeters of mercury, urine 500 to 800 cubic centimeters for twenty-four hours with a trace of albumin and many casts. No edema or fluid in cavities. Non-protein nitrogen, 28 milligrams in 100 cubic centimeters of blood.

The drowsiness deepened into stupor and after two days, coma. Death occurred on the fifth day after admission. Clinical diagnosis, uremia. Anatomical diagnosis, chronic nephritis (extremely small granular kidneys), edema of brain, marked colitis, uremia.

In this case it is evident that had undue weight been given to the low non-protein nitrogen one would have been led to conclude that there was not a severe nephritis, and that the retinal condition was due to some intracranial disease. Moreover, after the diagnosis of uremia had been made the non-protein nitrogen would have pointed, according to Widal, to a favorable outcome.

Tests and methods of investigation such as those I have discussed are undoubtedly valuable. Correctly applied they stimulate interest and lead in the long run to broadened knowledge. But just so soon as such methods are regarded as ultimate criteria, we are led first into errors in diagnosis, and from that creep into narrowed medicine and false ideas as to the pathology of a disease entity.

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2. Widal, Fernand: *Le Mouvement Medical*, 1913, i, 1.



## BLOOD-PRESSURE STUDIES IN TUBERCULOSIS AT A HIGH ALTITUDE. REPORT OF SIX HUNDRED CASES \*

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Six years ago one of us (Peters) began the study of blood-pressure in tuberculous individuals living under sanatorium conditions at an elevation of 6,000 feet. A preliminary report<sup>1</sup> was made in 1908 covering a series of one hundred cases. The findings at that time were in such marked contrast to the work of other observers, notably Gardiner and Hoagland<sup>2</sup> of Colorado Springs, that we decided to continue our observations until we had obtained readings in a sufficient number of cases to make the conclusions of some value from a scientific standpoint.

With this idea in view we have made records covering a series of six hundred cases, and are gratified to note that the conclusions reached in our earlier report are even more strongly borne out by a more exhaustive research.

The question of blood-pressure in tuberculosis has usually been dismissed with the dogmatic statement that the disease tends toward hypotension. The observations of workers at sea level have confirmed this statement many times over, but on the other hand, little work has been done along the same line at elevations considered especially adapted for the treatment of consumption. True, the work of Gardiner and Hoagland<sup>2</sup> already cited tends to show that altitude lowers blood-pressure, but the number of cases reported is far too small to allow of radical conclusions. Also, a number of the experiments were made on the top of Pike's Peak, at an elevation of over 14,000 feet, and from the difference in elevation alone would be of little value in a comparison with our results. We are willing to grant that on coming to a high altitude the pressure drops temporarily, but on residence a gradual rise is noted. This very fact, the sudden change from a moderate elevation to an altitude of over 14,000 feet, may probably account for the difference in our conclusions and those of Gardiner and Hoagland.

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\* Submitted for publication July 18, 1913.

\* Read in the Section on Practice of Medicine of the American Medical Association at the Sixty-Fourth Annual Session, held at Minneapolis, June, 1913.

\* From the laboratory of the New Mexico Cottage Sanatorium.

1. Peters, L. S.: *THE ARCHIVES INT. MED.*, 1908, ii, 42.

2. Gardiner and Hoagland: *Trans. Am. Climatol. Assn.*, 1905.

Now let us consider the work of Crile.<sup>3</sup> By means of a pneumatic cabinet he was able to show a rise in pressure when the surrounding atmospheric pressure was decreased. Along the same line may be mentioned the work of Lazarus and Schirmunski,<sup>4</sup> Bert<sup>5</sup> and many other observers. F. H. Bartlett<sup>6</sup> has investigated the effects of breathing rarified air in rabbits and finds that the aortic pressure falls with that of the inspired air after a sufficient negative pressure has been produced. Bert,<sup>5</sup> however, says that the technical difficulties render exact experiments in this respect impossible. Coagulation in the connecting piece between artery and manometer will always take place.

Mosso<sup>7</sup> made his studies on the top of Mount Rosa at an altitude of 4,560 meters. He found very little difference in readings at this elevation and sea level. Here again we make the same criticism of too high altitude. However, Mosso cites the work of K. Heller, W. Mayer and H. von Schroetter,<sup>8</sup> who, at an altitude of 2,210 meters, found a rise in pressure. This latter elevation more nearly coincides with the altitude at which our results were obtained. Further references could be given, such as the work of Frankel and Geppert,<sup>9</sup> who found that the pressure did not change with very considerable fall in atmospheric pressure even when of a nature dangerous to life. In one experiment in which the barometer showed 214 millimeters there was an increased blood-pressure and slowing of the pulse.

Liebig<sup>10</sup> states that results of observations on the effect of barometric pressure on blood-pressure are not uniform. He found that with a fall in atmospheric pressure the blood-pressure rose in three people, in one at a pressure corresponding to 2,800 meters altitude; in another under atmospheric pressure at 520 mm., and in a third in a pressure corresponding to 3,400 meters altitude. In a more extensive fall of atmospheric pressure, or otherwise a much higher altitude, the blood-pressure fell. This latter seems to coincide with the observations of Gardiner and Hoagland<sup>2</sup> in our country, namely, that in too high an elevation we get a reduction in blood-pressure.

The criticism we wish to make on all observations to which we have thus far referred is briefly stated thus: 1. The number of experiments and the number of people experimented on are far too small to warrant of any radical conclusions. 2. No comparison can be made between

3. Crile, Geo. W.: *Blood-Pressure in Surgery*, Phila., 1903, p. 283.

4. Lazarus and Schirmunski: *Ztschr. f. Klin. Med.*, 1884, vii.

5. Bert: *La Pression Barometrique*, Paris, 1878.

6. Bartlett, F. H.: *Am. Jour. Physiol.*, 1903, x, 143.

7. Mosso, A.: *Arch. ital. di biol.*, 1905, xliii, 341.

8. Heller, K., Mayer, W., and von Schroetter, H.: *Ztschr. f. klin. Med.*, xxxiii.

9. Frankel and Geppert: *Ueber die Wirkungen der verdunnten Luft. auf den Organismus*, Berlin, 1883, p. 65.

10. Liebig: *Sitzungb. d. Gesellsch. d. Morphol. u. Biol.*, 1896, xii.

observations on animals under pneumatic cabinet conditions and the observations on tuberculous invalids at 6,000 feet elevation. 3. No comparison can be made between observations made on man at extremely high elevations and on those made on tuberculous invalids at 6,000 feet elevation. 4. Pneumatic cabinet experiments on man cannot in any way prove or disprove the results of observations on tuberculous invalids residents of an elevation of 6,000 feet.

Referring now to the more recent work in this country of Haven Emerson.<sup>11</sup> He conducted a series of experiments on both man and animals to determine the causes of hypotension in tuberculous patients, and to determine the value of such symptoms as a diagnostic and prognostic measure. He concludes that the cause of hypotension is primarily a toxic action on the vasomotor center in the medulla, allowing of a vaso-paresis, or stimulating a vaso-dilatation, and secondarily, progressive cardiac atrophy or degeneration. He believes that hypotension is common enough in early cases to be sought for as carefully as is the custom at present to search for pulmonary signs. He draws about the same conclusion regarding prognosis as was contained in our earlier report,<sup>1</sup> namely, with betterment of pulmonary condition the blood-pressure is increased; stationary patients show little or no change, and progressive patients show a decrease in blood-pressure findings.

Ritter<sup>12</sup> also bases much on the prognosis from the blood-pressure observations and his work is based on records made on ambulatory patients treated at the out-patient department of Rush Medical College.

These last two observers — Emerson and Ritter — were working at sea level and with no intention of making a comparative study of observations there and at high altitudes. With their work we must also refer to that of Thayer<sup>13</sup> of Baltimore, and we shall use the latter two reports in making our comparison of average pressure since Emerson gives no adequate figures.

Considering all classes of cases our results show that at an elevation of 6,000 feet the average pressure in consumptives is 132 mm. of mercury. Thayer's averages for the same class of patients at sea level is 103 mm. of mercury, and Ritter's is 110 mm. From observations made here on normal individuals we find the average pressure to be 143, all ages considered from 19 to 66. Even this is a higher average than the majority of observers give for normal individuals at sea level.

On first coming to a high elevation the pressure is lowered, but after a short residence an increase is noted and in a majority of patients an increase over the original reading at sea level.

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11. Emerson, Haven: *THE ARCHIVES INT. MED.*, 1911, vii, 441.

12. Ritter, John: *Trans. Nat. Assn. for the Study and Prevention of Tuberculosis*, 1911, p. 297.

13. Thayer, Wm.: Cited by Stanton, *Internat. Clinics*, 1907, iii, Series 17.

From a prognostic standpoint we believe the blood-pressure findings are of great value in tuberculosis. Here, we have noted that if the patient continues to improve his pressure gradually rises. If no improvement is noted, but on the other hand, the status is stationary, the pressure remains practically the same. If the patient's condition grows worse there is a decrease in the blood-pressure reading.

We find also that the degree of involvement bears no relation to the pressure, and for that reason we have omitted a reference in the general table to the Turban classification and have classified our cases from the standpoint of toxemia. We note that a marked relation exists between blood-pressure and the degree of toxemia. Here also the inverse ratio of pulse and pressure noted by so many observers is clearly shown — a high pulse accompanied by a low pressure, and a low pulse by a normal or higher pressure.

We have also studied, in a limited number of cases, the relation of blood-pressure and pulmonary hemorrhage. Although we have been unable to observe readings immediately before the hemorrhage occurred, we know from records taken immediately after, that the pressure is increased from 15 to 25 mm. of mercury. It is only reasonable to suppose that such increase occurred before the hemorrhage and was the immediate cause of the bleeding. These cases are so few in our experience that we have not incorporated them as such in our table, but merely state the facts as we have seen them, hoping to gain some information in the future and be able to substantiate our observations by a sufficient number of cases to warrant our conclusions. Just why we have so few hemorrhages at this elevation with our increased pressure is an open question. The two facts seem to be in direct contradiction. However, the lowered blood-pressure on arrival, together with a relaxed circulatory system, gives the body time to accommodate itself to the change, and owing to the stimulating effect of altitude on cardiac and vascular tone the walls of the capillaries and circulatory system in general are strengthened and are better able to stand the increased pressure which afterwards results. It is a well-known fact that patients bleeding at sea level over a long period of time (having been placed on a train during this series), on arrival in the elevation of the southwest have been surprised to find such hemorrhages cease and never occur again.

Heretofore the knowledge of climate in the treatment of tuberculosis was empirical. Consumptives were sent west and made a recovery, or were buried on the plains and hillsides in a strange country. The laity and physicians have granted from time immemorial the value of climate in the treatment of tuberculosis. Some early observer before the time of Hippocrates, wisely said: "If you would get well of consumption go into the mountains and live on the fruit of the cow," and opinion has



TABLE 1.—DATA CONCERNING SIX HUNDRED CONSUMPTIVES

Case No.	Age	Sex	Blood Pressure		Pulse Av.	Temp. F. Av.	Urine	Degree of Toxemia	Status on Discharge
			Ad.	Av.					
1	32	M	106	126	80	98.6	N	—	Arrested
2	18	F	101	109	90	98.6	N	—	Improved
3	25	M	102	118	80	98.6	N	—	Improved
4	27	M	112	124	76	98.6	Alb. Casts	—	Improved
5	27	M	100	91	80	100.0	N	++	Progressive
6	27	M	104	98	140	102.0	Alb. Casts	+++	Progressive
7	43	F	130	130	?	99.0	N	+	?
8	22	F	108	116	85	98.0	N	—	App. Cured
9	28	M	125	142	80	99.0	Casts	+	Arrested
10	34	M	113	116	84	98.6	N	—	Arrested
11	25	M	128	102	80	98.6	N	—	Unimproved
12	18	M	121	121	80	98.6	N	—	Unimproved
13	33	M	100	106	104	99.6	Alb. Casts	+	Progressive
14	54	M	132	126	100	98.6	N	—	Unimproved
15	29	M	112	100	100	101.0	N	+++	Progressive
16	47	M	126	148	84	98.6	N	—	Arrested
17	24	F	103	119	70	98.6	N	—	App. Cured
18	29	M	120	130	90	99.0	N	+	Improved
19	32	M	94	90	84	98.6	N	—	Arrested
20	23	F	104	104	80	98.6	N	—	Improved
21	23	M	104	134	75	98.6	N	—	Arrested
22	35	M	112	112	102	98.6	Alb.	—	Improved
23	22	M	110	120	98	98.6	N	—	Arrested
24	37	M	112	124	80	98.6	N	—	Arrested
25	24	M	117	117	128	98.6	N	—	Unimproved
26	25	M	130	132	74	98.6	N	—	App. Cured
27	28	F	119	120	80	98.6	N	—	Improved
28	44	M	135	144	104	98.6	N	—	Arrested
29	37	M	102	110	90	98.6	N	—	Improved
30	42	M	118	110	100	98.6	N	—	Improved
31	30	M	120	150	100	98.6	N	—	Improved
32	28	M	134	176	80	99.4	Alb. Casts	+	Unimproved*
33	28	M	110	126	120	98.6	N	—	Arrested
34	40	M	131	144	90	99.0	Alb	+	Arrested
35	48	M	114	120	75	98.6	Casts	—	Arrested
36	29	M	86	120	76	98.6	N	—	App. Cured
37	25	M	122	138	88	98.6	N	—	App. Cured
38	19	F	122	126	90	98.6	N	—	Improved
39	27	M	122	130	80	99.0	Casts	+	Improved
40	22	M	134	144	90	98.6	Casts	—	Arrested
41	29	M	138	140	90	98.6	Casts	—	Arrested
42	25	M	90	124	100	99.8	N	+	Improved
43	23	M	120	110	90	98.6	Casts	—	Progressive
44	28	M	110	110	85	98.6	N	—	Unimproved
45	21	M	106	118	132	99.2	Alb. Casts	+	Improved
46	40	M	130	172	95	99.0	Casts	+	Arrested
47	25	M	134	130	90	98.6	N	—	Arrested
48	40	M	160	158	95	100.0	Casts	++	Unimproved
49	34	M	96	110	80	98.6	Casts	—	App. Cured
50	23	M	112	118	90	98.6	Casts	—	Improved
51	52	F	122	140	84	98.6	N	—	App. Cured
52	24	M	130	142	90	98.6	Casts	—	Improved
53	58	M	110	126	92	98.6	N	—	App. Cured

\* Kidneys responsible for high blood-pressure.

TABLE 1.—DATA CONCERNING SIX HUNDRED CONSUMPTIVES—(Continued)

Case No.	Age	Sex	Blood Pressure		Pulse Av.	Temp. F. Av.	Urine	Degree of Toxemia	Status on Discharge
			Ad.	Av.					
54	29	F	130	130	84	98.6	Casts	—	Improved
55	28	M	110	100	112	99.4	Casts	+	Progressive
56	23	M	130	128	70	99.0	Casts	+	Improved
57	52	M	164	150	138	99.0	Alb. Casts	+	Unimproved
58	49	M	136	144	100	98.6	Alb. Casts	—	Arrested
59	29	M	120	120	120	100.0	N	++	Improved
60	28	M	110	130	108	98.6	Casts	—	App. Cured
61	35	F	128	130	108	98.6	N	—	Improved
62	33	F	110	102	110	98.6	N	—	Progressive
63	22	F	110	124	92	98.6	N	—	Improved
64	31	M	98	140	100	98.6	N	—	Improved
65	48	M	134	140	84	99.1	Alb. Casts	+	Arrested
66	24	M	128	134	85	98.6	Casts	—	Improved
67	42	F	110	126	120	99.0	Alb. Casts	+	Improved
68	40	M	130	148	80	98.6	Casts	—	Arrested
69	36	F	150	150	100	98.6	N	—	App. Cured
70	27	F	106	120	88	98.6	Casts	—	App. Cured
71	38	M	88	110	80	99.4	Casts	+	Improved
72	27	M	119	128	75	98.6	N	—	Arrested
73	32	M	110	128	75	98.6	N	—	Arrested
74	24	M	100	126	80	98.6	Casts	—	Arrested
75	30	F	110	140	80	98.6	N	—	App. Cured
76	40	M	106	118	100	99.2	N	+	Improved
77	46	M	134	150	80	98.6	N	—	Improved
78	35	M	148	160	85	99.0	Alb. Casts	+	Unimproved
79	35	M	118	140	75	99.5	N	+	Improved
80	35	M	122	122	110	98.6	N	—	Unimproved
81	29	M	130	134	80	98.6	N	—	Improved
82	45	M	142	148	120	99.0	N	+	Arrested
83	42	M	146	154	80	98.6	Casts	—	Improved
84	26	M	96	130	80	98.6	N	—	Improved
85	25	M	110	120	80	98.6	N	—	App. Cured
86	22	M	88	112	75	98.6	N	—	Arrested
87	28	F	112	112	100	98.6	Casts	—	Progressive
88	39	M	113	120	100	98.6	N	—	Arrested
89	28	M	102	126	85	98.6	N	—	Arrested
90	40	F	108	108	90	100.0	Alb. Casts	+++	Progressive
91	37	M	114	134	85	98.6	N	—	Arrested
92	25	M	108	124	85	98.6	N	—	Arrested
93	23	M	128	128	75	98.6	Casts	—	App. Cured
94	26	M	130	135	85	98.6	N	—	Arrested
95	36	F	102	122	90	98.6	N	—	Arrested
96	34	M	106	116	120	99.4	N	+	Improved
97	47	M	108	120	100	99.4	Alb. Casts	+	Improved
98	18	F	128	136	100	98.6	N	—	Improved
99	27	F	130	138	88	98.6	N	—	Arrested
100	28	M	114	146	80	98.6	Alb. Casts	—	Improved
101	26	M	125	125	90	101.0	N	+++	.....†
102	23	M	125	145	90	99.0	N	+	Arrested
103	33	M	100	116	90	99.6	N	+	Improved
104	41	M	140	150	85	99.2	Hyn. Casts	+	App. Cured
105	28	M	130	130	85	98.6	Casts	—	Progressive
106	23	M	125	130	90	98.6	Casts	—	App. Cured
107	29	F	118	130	80	99.2	N	+	App. Cured

† Died following hemorrhage.

TABLE 1.—DATA CONCERNING SIX HUNDRED CONSUMPTIVES—(Continued)

Case No.	Age	Sex	Blood Pressure		Pulse Av.	Temp. F. Av.	Urine	Degree of Toxemia	Status on Discharge
			Ad.	Av.					
108	26	M	120	130	70	98.6	N	—	Arrested
109	25	M	125	160	90	100.5	N	++	Improved
110	37	M	120	125	100	100.0	N	++	Progressive
111	26	M	124	150	90	99.2	N	+	Arrested
112	32	M	125	150	100	100.0	N	++	App. Cured
113	30	F	128	128	85	98.6	N	—	Arrested
114	33	M	125	140	85	98.6	N	—	Arrested
115	21	M	120	145	90	99.0	N	+	Arrested
116	28	M	100	100	120	101.0	Casts	+++	Progressive
117	28	M	130	135	80	98.6	N	—	Arrested
118	23	M	120	130	74	99.0	N	+	Improved
119	23	F	110	135	90	98.6	N	—	App. Cured
120	26	M	132	145	85	98.6	N	—	Arrested
121	17	M	100	125	100	99.0	N	+	Arrested
122	23	M	118	135	90	99.4	N	+	Improved
123	21	M	125	150	90	99.6	N	+	Arrested
124	28	M	115	150	80	98.6	N	—	App. Cured
125	29	M	118	135	85	99.0	N	+	Improved
126	32	F	125	145	100	99.0	N	+	Arrested
127	24	M	130	150	75	98.6	N	—	App. Cured
128	36	M	135	140	80	98.6	N	—	Arrested
129	30	F	110	135	96	99.2	N	+	Arrested
130	21	F	110	135	90	98.6	N	—	App. Cured
131	40	F	116	136	100	99.4	N	+	Arrested
132	24	F	130	134	96	98.6	N	—	App. Cured
133	15	F	114	135	96	98.6	N	—	App. Cured
134	21	M	145	148	80	98.6	N	—	Arrested
135	44	M	150	130	100	99.5	N	+	Progressive
136	21	M	140	155	120	99.2	N	+	Improved
137	32	M	115	138	90	99.0	N	+	Arrested
138	39	M	130	105	100	99.4	N	+	Progressive
139	38	M	140	160	80	99.0	N	+	App. Cured
140	35	M	130	145	100	99.2	N	+	Improved
141	33	M	140	140	100	100.0	Alb.	++	Unimproved
142	56	M	140	150	80	98.6	N	—	Improved
143	37	M	130	145	88	98.6	N	—	App. Cured
144	58	M	185	165	120	98.6	Alb.	—	Progressive
145	44	F	133	135	88	98.6	N	—	App. Cured
146	25	F	115	125	90	100.0	N	++	Improved
147	29	M	130	140	80	98.6	N	—	App. Cured
148	22	M	142	155	76	98.6	N	—	App. Cured
149	27	M	135	125	90	99.2	N	+	Unimproved
150	55	M	160	160	80	98.6	N	—	Arrested
151	25	M	100	115	86	100.0	N	++	Arrested
152	36	M	135	120	100	100.0	N	++	Progressive
153	24	M	140	112	120	99.4	N	+	Progressive
154	30	M	125	130	76	98.6	N	—	App. Cured
155	19	M	120	130	88	99.6	N	+	Arrested
156	35	M	120	130	100	99.2	N	+	Improved
157	26	M	120	140	90	98.6	N	—	App. Cured
158	26	M	120	110	100	99.4	N	+	Arrested
159	36	M	115	130	100	99.0	N	+	Improved
160	26	M	110	88	120	101.0	N	+++	Died
161	30	F	130	120	95	100.0	N	++	Progressive
162	30	M	120	120	85	98.6	N	—	App. Cured

TABLE 1.—DATA CONCERNING SIX HUNDRED CONSUMPTIVES—(Continued)

Case No.	Age	Sex	Blood Pressure		Pulse Av.	Temp. F. Av.	Urine	Degree of Toxemia	Status on Discharge
			Ad.	Av.					
163	26	M	110	130	90	99.4	N	+	Improved
164	23	M	104	130	100	99.6	N	+	Arrested
165	32	F	113	130	95	99.2	N	+	App. Cured
166	22	M	105	120	90	98.6	N	—	Arrested
167	22	M	100	130	90	99.6	N	+	Improved
168	45	F	95	136	85	98.6	N	—	Arrested
169	19	F	110	120	80	98.6	N	—	Improved
170	25	M	94	125	100	98.6	N	—	Improved
171	32	F	103	125	90	98.6	N	—	App. Cured
172	29	M	126	130	95	99.4	N	+	Improved
173	25	F	100	126	100	99.0	N	+	Improved
174	40	F	120	135	85	98.6	N	—	Arrested
175	25	M	122	138	85	98.6	N	—	App. Cured
176	48	F	135	135	90	98.6	N	—	App. Cured
177	29	M	138	138	100	99.4	N	+	Improved
178	26	M	124	130	120	100.0	N	++	.....‡
179	31	M	120	115	100	100.0	N	++	.....‡
180	36	F	146	146	100	99.2	N	+	Improved§
181	40	M	150	138	90	99.6	Sugar	+	Improved
182	26	M	128	132	85	98.6	N	—	App. Cured
183	37	M	124	135	85	99.2	N	+	Improved
184	18	M	122	135	75	98.6	N	—	App. Cured
185	22	M	140	140	120	99.2	N	+	Unimproved
186	34	M	134	118	100	99.4	N	+	Progressive
187	20	M	134	145	90	99.0	N	+	Improved
188	24	F	110	118	90	99.6	N	+	Improved
189	18	F	118	118	100	99.4	N	+	Progressive
190	27	M	116	145	90	99.8	N	+	Progressive¶
191	35	F	130	125	90	99.0	N	+	Improved
192	35	M	125	140	90	98.6	N	—	Arrested
193	23	M	116	116	120	101.0	N	+++	Progressive
194	24	F	130	130	90	98.6	N	—	App. Cured
195	20	M	142	142	90	98.6	N	—	App. Cured
196	43	F	105	128	90	98.6	N	—	App. Cured
197	42	M	126	138	85	98.6	N	—	Arrested
198	27	M	138	145	84	98.6	N	—	.....‡
199	32	F	132	126	100	100.0	N	++	.....‡
200	18	F	126	126	80	98.6	N	—	Arrested
201	28	M	125	125	110	99.6	N	+	Unimproved
202	25	M	132	130	85	98.6	N	—	Improved
203	36	M	176	150	80	99.0	N	+	Arrested
204	25	M	132	145	90	98.6	N	—	Arrested
205	26	M	132	130	85	98.6	N	—	App. Cured
206	19	M	122	110	120	101.0	N	+++	Progressive
207	33	M	110	120	100	99.4	N	+	Improved
208	26	M	124	120	80	100.0	N	++	Progressive
209	20	M	108	135	85	99.0	N	+	Improved
210	25	M	120	123	75	98.6	N	—	App. Cured
211	28	F	90	108	100	98.6	N	—	Arrested
212	26	M	105	120	100	99.6	N	+	Improved
213	20	M	105	100	90	100.0	N	++	Progressive

‡ Died suddenly. Acute dilatation of heart.

§ Sugar responsible for high blood-pressure.

¶ Hemorrhage case. High blood-pressure due to this.

|| Alcoholic.



TABLE 1.—DATA CONCERNING SIX HUNDRED CONSUMPTIVES — (Continued)

Case No.	Age	Sex	Blood Pressure		Pulse Av.	Temp. F. Av.	Urine	Degree of Toxemia	Status on Discharge
			Ad.	Av.					
214	38	M	133	145	80	98.6	N	—	Arrested
215	17	F	122	127	85	98.6	N	—	App. Cured
216	27	M	118	130	85	98.6	N	—	Arrested
217	28	M	110	123	80	98.6	N	—	App. Cured
218	30	F	105	120	80	98.6	N	—	Arrested
219	21	M	110	120	100	98.6	Trace Alb.	—	Improved
220	39	F	110	135	100	99.4	N	+	Improved
221	32	F	95	97	98	98.6	N	—	Arrested
222	20	F	126	127	75	99.0	N	+	Arrested
223	30	F	120	124	90	98.6	N	—	Arrested
224	27	F	100	108	100	99.6	N	+	.....†
225	40	M	120	145	100	98.6	N	—	Arrested
226	21	M	120	124	90	98.6	N	—	Improved
227	47	M	118	140	95	99.0	N	+	Arrested
228	33	M	120	120	90	98.6	N	—	Arrested
229	60	F	115	145	100	98.6	N	—	App. Cured
230	30	M	120	148	80	98.6	N	—	Arrested
231	19	M	120	130	90	98.6	N	—	Arrested
232	18	M	107	118	95	100.0	N	++	Progressive
233	25	M	125	133	85	98.6	N	—	Arrested
234	25	M	110	110	95	100.0	N	++	Improved
235	27	F	120	120	90	98.6	N	—	Arrested
236	24	M	122	127	85	98.6	N	—	App. Cured
237	18	M	125	138	100	99.0	N	+	Improved
238	39	M	115	130	100	99.0	N	+	Unimproved
239	23	M	120	120	100	99.0	N	+	Improved
240	41	F	100	115	100	101.0	N	+++	Improved
241	26	F	115	125	75	98.6	N	—	App. Cured
242	36	M	124	115	88	99.6	N	+	Progressive
243	29	M	122	138	90	99.0	N	+	Improved
244	36	M	122	123	90	99.0	N	+	Improved
245	24	F	128	130	90	99.0	N	+	Improved
246	26	M	115	125	80	98.6	N	—	App. Cured
247	29	M	100	100	120	100.0	N	++	Progressive
248	41	M	122	112	120	100.0	N	++	Progressive
249	20	F	110	115	100	98.6	N	—	App. Cured
250	39	M	128	130	80	98.6	N	—	App. Cured
251	32	M	130	135	85	98.6	N	—	Arrested
252	36	M	118	110	120	100.0	N	++	Progressive
253	57	M	132	140	110	100.0	Alb.	++	Progressive
254	22	M	110	118	110	98.6	N	—	App. Cured
255	44	M	105	120	85	99.0	Alb.	+	Arrested
256	39	F	120	120	90	98.6	N	—	Improved
257	35	F	102	112	90	98.6	N	—	Improved
258	28	F	110	114	..	98.6	N	—	App. Cured
259	49	M	110	120	80	99.6	N	+	Improved
260	42	M	123	126	95	98.6	N	—	Arrested
261	39	M	120	125	80	98.6	N	—	App. Cured
262	23	M	117	119	85	98.6	N	—	Arrested
263	22	M	130	115	100	99.8	N	+	Progressive
264	29	F	130	130	80	98.6	N	—	App. Cured
265	48	M	135	138	80	98.6	N	—	App. Cured
266	31	M	104	115	90	99.6	N	+	Improved
267	32	M	124	150	90	98.6	N	—	Arrested
268	26	M	125	118	110	100.0	N	++	Progressive

TABLE 1.—DATA CONCERNING SIX HUNDRED CONSUMPTIVES — (Continued)

Case No.	Age	Sex	Blood Pressure		Pulse Av.	Temp. F. Av.	Urine	Degree of Toxemia	Status on Discharge
			Ad.	Av.					
269	22	M	122	130	100	99.0	N	+	Improved
270	29	M	125	128	..	98.6	N	—	App. Cured
271	38	M	124	124	120	100.4	N	++	Progressive
272	20	F	120	120	90	99.0	N	+	Unimproved
273	35	F	155	160	95	99.6	N	+	App. Cured
274	25	F	125	126	80	98.6	N	—	App. Cured
275	43	F	125	125	90	98.6	N	—	Unimproved
276	44	M	122	135	..	98.6	N	—	App. Cured
277	33	M	125	140	90	98.6	N	—	App. Cured
278	25	M	128	135	80	98.6	N	—	Improved
279	30	F	125	125	85	98.6	N	—	Unimproved
280	43	M	126	127	85	99.0	N	+	Unimproved
281	47	F	103	125	100	99.4	N	+	Arrested
282	26	M	118	119	90	99.4	N	+	Unimproved
283	42	F	108	105	120	100.0	N	++	Progressive
284	42	F	120	125	100	98.6	N	—	Improved
285	22	F	118	120	95	98.6	N	—	Arrested
286	33	M	119	123	105	99.0	N	+	Arrested
287	22	M	125	125	90	99.4	Trace Alb.	+	Unimproved
288	23	F	98	123	90	98.6	N	—	Arrested
289	21	M	115	112	120	102.0	N	+++	Progressive
290	26	F	98	118	110	99.0	N	+	Arrested
291	19	M	112	112	130	99.6	N	+	Progressive
292	22	M	123	120	120	101.0	N	+++	Progressive**
293	20	M	108	115	95	99.0	N	+	Progressive
294	37	M	122	112	110	100.0	N	++	Progressive
295	6	F	90	100	90	99.0	N	+	App. Cured††
296	31	M	135	138	95	98.6	N	—	Improved
297	40	M	130	135	85	98.6	N	—	Arrested
298	40	F	137	137	85	99.0	N	+	Unimproved
299	23	M	116	115	90	99.0	N	+	Unimproved
300	36	M	134	145	80	98.6	N	—	App. Cured
301	42	F	110	110	120	99.2	Alb. Casts	+	Progressive
302	18	F	128	135	80	99.0	N	+	App. Cured
303	32	M	115	112	90	Nor.	N	—	Arrested
304	35	M	130	140	100	Nor.	N	—	Arrested
305	30	F	125	125	85	Nor.	N	—	App. Cured
306	35	M	120	130	90	99.0	N	+	Arrested
307	22	M	100	120	90	Nor.	N	—	App. Cured
308	22	M	105	125	95	Nor.	N	—	App. Cured
309	22	M	100	125	90	Nor.	N	—	Arrested
310	32	F	103	120	90	Nor.	N	—	App. Cured
311	25	F	100	120	100	Nor.	N	—	Improved
312	24	F	95	115	90	99.2	Alb. Casts	+	App. Cured
313	26	F	105	110	130	102.0	Casts	+++	Progressive
314	19	M	130	140	90	Nor.	Casts	—	App. Cured††
315	33	M	137	140	80	Nor.	N	—	App. Cured
316	39	F	100	125	90	Nor.	N	—	Arrested
317	23	M	110	130	95	99.2	N	+	Improved
318	18	M	107	107	90	99.3	N	+	Stationary
319	45	M	125	115	90	99.2	N	+	Progressive
320	20	F	115	120	96	99.0	N	+	Improved

\*\* Blood-pressure not taken toward end of treatment.

†† Joint case.

‡‡ Urine normal on discharge.

TABLE 1.—DATA CONCERNING SIX HUNDRED CONSUMPTIVES—(Continued)

Case No.	Age	Sex	Blood Pressure		Pulse Av.	Temp. F. Av.	Urine	Degree of Toxemia	Status on Discharge
			Ad.	Av.					
321	21	M	152	160	85	Nor.	N	—	Arrested
322	33	M	128	145	100	99.3	N	+	Improved
323	34	M	124	127	90	Nor.	N	—	Improved
324	25	F	100	125	80	Nor.	N	—	App. Cured
325	27	M	130	140	85	Nor.	N	—	Arrested
326	26	M	138	140	85	Nor.	N	—	App. Cured
327	41	M	104	125	90	99.2	N	+	Improved
328	35	M	104	117	90	99.0	N	—	Died§§
329	47	F	148	145	100	100.0	N	++	Progressive
330	33	M	150	145	80	99.0	N	+	Arrested
331	26	M	134	142	80	Nor.	N	—	App. Cured
332	34	F	105	110	88	100.0	N	++	Improved
333	30	M	128	134	80	Nor.	N	—	App. Cured
334	36	M	134	140	80	Nor.	N	—	App. Cured
335	30	M	114	120	100	100.4	N	++	Improved
336	21	M	104	134	85	Nor.	N	—	Arrested
337	32	F	135	135	90	99.4	N	+	Arrested
338	27	M	125	128	88	Nor.	N	—	App. Cured
339	24	F	98	90	120	100.0	N	++	Progressive
340	22	M	120	124	85	Nor.	N	—	App. Cured
341	23	M	100	130	90	Nor.	N	+	App. Cured
342	45	F	145	150	90	99.0	N	+	Arrested
343	18	F	120	125	80	99.0	N	+	App. Cured
344	29	M	124	145	72	99.0	N	—	Improved
345	25	M	138	150	90	Nor.	N	—	Arrested
346	51	M	128	144	90	Nor.	N	—	Improved
347	32	F	120	135	80	Nor.	N	—	App. Cured
348	18	F	115	125	85	Nor.	N	—	App. Cured
349	21	M	124	140	120	99.4	N	+	Improved
350	32	F	100	138	85	Nor.	N	—	Arrested
351	30	M	140	144	80	Nor.	N	—	App. Cured
352	32	M	124	134	80	Nor.	N	—	App. Cured
353	25	F	130	136	80	Nor.	N	—	Arrested
354	45	M	154	154	100	99.0	N	+	Stationary
355	24	M	144	154	90	Nor.	N	—	Arrested
356	31	M	144	150	75	Nor.	N	—	App. Cured
357	39	M	145	126	125	99.3	N	+	Progressive
358	23	M	128	150	75	Nor.	N	—	App. Cured
359	23	M	115	140	80	Nor.	N	—	App. Cured
360	22	M	122	130	80	Nor.	N	—	App. Cured
361	22	F	100	118	80	Nor.	N	—	App. Cured
362	18	M	140	144	80	Nor.	N	—	Arrested
363	32	M	120	140	85	Nor.	N	—	Arrested
364	34	F	124	135	95	Nor.	N	—	Improved
365	24	F	128	135	85	Nor.	N	—	App. Cured
366	22	F	118	124	72	Nor.	N	—	App. Cured
367	39	F	114	120	90	Nor.	N	—	Arrested
368	23	F	120	120	100	100.0	N	++	Progressive
369	24	M	108	125	95	Nor.	N	—	Improved
370	28	M	150	160	72	Nor.	N	—	App. Cured
371	19	M	146	146	72	Nor.	N	—	App. Cured
372	41	F	135	140	80	Nor.	N	—	App. Cured
373	28	M	114	125	90	100.0	N	++	Improved
374	31	F	124	135	90	99.0	N	+	Improved
375	20	M	122	150	100	99.0	N	+	Improved
376	22	M	110	135	95	99.2	N	+	Improved

§§ Sudden hemorrhage.

TABLE 1.—DATA CONCERNING SIX HUNDRED CONSUMPTIVES — (Continued)

Case No.	Age	Sex	Blood Pressure		Pulse Av.	Temp. F. Av.	Urine	Degree of Toxemia	Status on Discharge
			Ad.	Av.					
377	25	M	128	150	95	Nor.	N	—	Improved
378	40	F	128	128	100	100.0	N	++	Stationary
379	32	M	134	110	90	99.4	N	+	Progressive
380	22	F	116	125	78	99.0	N	+	Improved
381	25	M	110	125	85	Nor.	N	—	Improved
382	28	M	138	150	100	Nor.	N	—	Improved
383	35	M	124	140	90	Nor.	N	—	Improved
384	44	F	98	115	90	Nor.	N	—	Improved
385	23	M	116	135	85	99.0	N	+	Improved
386	23	M	145	145	90	99.0	N	+	Improved
387	27	M	126	130	100	99.2	N	+	Improved
388	25	M	134	140	85	Nor.	N	—	Arrested
389	29	F	114	125	90	Nor.	N	—	Improved
390	24	M	116	125	95	99.0	N	+	Improved
391	31	F	135	140	80	Nor.	N	—	App. Cured
392	20	M	148	135	85	Nor.	Alb. Casts	—	Improved <sup>¶¶</sup>
393	23	M	100	128	72	Nor.		—	Arrested
394	21	M	145	145	85	Nor.	N	—	Improved
395	25	M	150	150	72	Nor.	N	—	Improved
396	27	M	146	150	72	Nor.	N	—	Improved
397	38	M	130	135	70	Nor.	N	—	Improved
398	18	M	104	115	110	100.0	N	++	Stationary
399	39	M	125	138	90	Nor.	N	—	Improved
400	29	F	115	110	90	101.0	N	+++	Stationary
401	29	F	135	140	78	Nor.	N	—	Arrested
402	29	M	148	148	80	Nor.	N	—	Improved
403	31	M	124	130	80	Nor.	N	—	Improved
404	30	F	120	130	90	99.3	N	+	Improved
405	24	F	118	122	90	99.0	N	+	Improved
406	20	M	126	135	90	Nor.	N	—	Arrested
407	23	F	130	135	90	99.0	N	+	Improved
408	35	M	145	155	80	Nor.	N	+	Arrested
409	23	M	134	150	85	99.0	N	+	Improved
410	19	M	144	160	85	Nor.	N	—	Arrested
411	26	M	132	140	100	99.1	N	+	Improved
412	36	M	176	188	90	Nor.	N	—	App. Cured <sup>¶¶¶</sup>
413	41	F	128	136	85	99.4	N	+	Improved
414	33	F	124	140	90	Nor.	N	—	Arrested
415	45	M	136	150	72	99.2	N	+	Improved
416	35	M	180	190	90	Nor.	N	—	Arrested
417	33	M	166	170	90	Nor.	N	—	Improved
418	28	M	140	140	72	Nor.	N	—	Arrested
419	21	M	138	142	72	Nor.	N	—	Improved
420	28	M	146	148	90	99.0	N	+	Improved
421	38	M	132	126	120	101.0	N	++	Progressive
422	45	M	148	140	95	99.0	N	+	Stationary
423	40	M	148	148	80	Nor.	N	—	Stationary
424	25	F	128	140	90	99.0	N	+	Improved
425	22	M	130	152	95	99.1	Alb. Casts	+	Improved
426	20	M	150	150	100	99.3		+	Stationary
427	41	M	152	146	90	Nor.	N	—	Improved
428	21	F	114	138	95	99.0	N	+	App. Cured
429	40	F	140	148	80	Nor.	N	—	App. Cured
430	22	M	160	150	120	99.0	N	+	Progressive

¶¶ Removal of tuberculous testicle.

¶¶¶ Hard drinker.



TABLE 1.—DATA CONCERNING SIX HUNDRED CONSUMPTIVES — (Continued)

Case No.	Age	Sex	Blood Pressure		Pulse Av.	Temp. F. Av.	Urine	Degree of Toxemia	Status on Discharge
			Ad.	Av.					
431	35	M	134	138	100	99.0	N	+	Stationary
432	29	M	138	138	85	Nor.	N	—	Improved
433	28	F	160	154	90	99.0	N	+	Improved
434	27	M	134	145	112	99.2	N	+	Improved
435	21	M	140	148	105	Nor.	N	—	Improved
436	26	M	148	160	80	Nor.	N	—	Arrested
437	20	M	145	136	72	Nor.	N	—	Arrested
438	33	F	122	148	90	99.0	N	+	Arrested
439	30	F	140	140	80	Nor.	N	—	Cured
440	20	M	132	136	100	100.3	Alb. Casts	++	Died†
441	25	M	120	140	120	99.2	N	+	Stationary
442	30	M	134	145	85	Nor.	N	—	Arrested
443	37	M	136	148	85	99.1	N	+	Cured
444	30	M	160	127	120	101.0	N	++	Progressive
445	30	M	150	155	85	Nor.	N	—	Arrested
446	27	M	118	125	100	99.0	N	+	Improved
447	31	F	134	140	90	99.2	N	+	Arrested
448	20	M	178	165	80	Nor.	N	—	Arrested
449	28	F	130	145	95	99.2	N	+	Improved
450	44	M	132	140	90	99.2	N	+	Improved
451	49	M	150	150	85	Nor.	N	—	Arrested
452	54	M	140	175	90	99.0	N	+	Arrested
453	33	M	148	155	72	Nor.	N	—	Arrested
454	24	M	175	175	85	Nor.	N	—	Arrested
455	33	M	130	140	80	Nor.	N	—	App. Cured
456	19	M	140	155	85	99.0	N	+	Arrested
457	27	M	130	126	100	99.3	N	+	Progressive
458	36	M	138	138	95	99.0	N	+	Arrested
459	58	M	122	122	130	101.0	N	++	Progressive
460	26	M	150	150	80	99.0	N	+	App. Cured
461	23	M	110	135	90	99.0	N	+	Improved
462	23	M	130	125	95	99.3	N	+	Progressive
463	33	M	136	145	100	Nor.	N	—	Arrested
464	22	M	128	145	85	Nor.	N	—	App. Cured
465	21	M	122	146	80	Nor.	N	—	Arrested
466	26	M	140	160	85	Nor.	N	—	Arrested
467	24	M	132	145	90	99.2	N	+	Improved
468	51	F	142	148	80	Nor.	N	—	App. Cured
469	30	M	130	135	90	Nor.	N	—	Improved
470	37	M	142	146	90	Nor.	N	—	Arrested
471	21	M	130	140	100	100.0	N	+	Improved
472	22	M	128	135	100	99.0	N	+	Improved
473	38	M	144	148	110	100.0	Sug. & Alb.	+	Prog'sive***
474	26	M	140	150	80	Nor.	N	—	Arrested
475	22	F	130	135	100	Nor.	N	—	Arrested
476	26	M	132	140	85	99.0	N	+	App. Cured
477	38	M	142	155	85	Nor.	N	—	App. Cured
478	30	M	114	126	80	99.2	N	+	Improved
479	25	M	140	140	90	Nor.	N	—	Arrested
480	39	M	128	136	100	99.4	N	+	Improved
481	37	M	140	142	85	Nor.	N	—	Arrested
482	17	M	142	148	90	99.0	N	+	Arrested
483	23	M	100	100	120	101.0	Alb. Casts	++	Progressive
484	27	M	150	150	90	Nor.	N	—	Arrested
485	20	F	138	140	85	99.0	N	+	Arrested

\*\*\* Kidney case.

TABLE 1.—DATA CONCERNING SIX HUNDRED CONSUMPTIVES — (Continued)

Case No.	Age	Sex	Blood Pressure		Pulse Av.	Temp. F. Av.	Urine	Degree of Toxemia	Status on Discharge
			Ad.	Av.					
486	50	M	110	120	100	100.0	N	+	Improved
487	23	M	146	155	85	Nor.	N	—	Arrested
488	27	M	118	135	80	Nor.	N	—	Arrested
489	21	F	130	135	100	99.2	N	+	Improved
490	18	F	140	140	100	99.2	N	+	Improved
491	24	M	122	145	72	Nor.	N	—	App. Cured
492	26	M	145	145	90	Nor.	N	—	Arrested
493	46	M	160	155	90	99.2	N	+	Progressive
494	44	M	122	140	100	Nor.	N	—	Arrested
495	26	M	118	95	110	100.2	N	++	Progressive
496	33	M	106	120	100	99.4	N	+	Improved
497	32	M	116	130	100	99.2	N	+	Improved
498	26	M	130	120	100	100.0	N	+	Progressive
499	27	M	142	142	90	Nor.	N	—	Cured
500	26	M	136	144	95	Nor.	N	—	Arrested
501	33	M	112	106	120	100.0	N	+	Progressive
502	24	F	128	128	120	101.0	N	++	Progressive
503	36	M	142	145	90	99.4	N	+	Improved
504	26	M	150	150	80	Nor.	N	—	Arrested
505	50	M	100	130	90	Nor.	N	—	Arrested
506	30	F	130	150	90	Nor.	N	—	Arrested
507	29	M	136	140	90	Nor.	N	—	Improved
508	26	M	126	135	72	Nor.	N	—	Improved
509	19	F	124	124	90	Nor.	N	—	Improved
510	22	M	127	138	90	Nor.	N	—	Improved
511	30	F	130	112	100	100.0	N	+	Progressive
512	27	F	145	150	85	Nor.	N	—	Improved
513	22	F	132	135	85	99.0	N	+	Improved
514	25	M	125	125	72	Nor.	N	—	Stationary
515	34	F	125	130	90	99.2	N	+	Arrested
516	22	M	124	145	90	99.0	N	+	Arrested
517	43	M	100	140	90	99.4	N	+	Improved
518	34	M	134	144	85	Nor.	N	—	App. Cured
519	35	F	110	130	90	Nor.	N	—	Arrested
520	23	F	132	138	80	Nor.	N	—	Improved
521	36	M	150	155	90	Nor.	N	—	Improved
522	30	M	126	128	90	Nor.	N	—	Improved.
523	24	M	125	125	90	Nor.	N	—	Stationary
524	40	F	135	140	80	99.2	N	+	Arrested
525	29	F	125	130	90	99.0	N	+	Improved
526	34	M	138	145	80	Nor.	N	—	Arrested
527	22	M	135	145	85	Nor.	N	—	Improved
528	33	M	134	150	72	Nor.	N	—	Improved
529	27	M	124	135	90	Nor.	N	—	Arrested
530	24	F	116	125	90	Nor.	N	—	Improved
531	32	F	120	125	85	99.2	N	+	Improved
532	51	M	125	140	90	Nor.	N	—	Improved
533	29	M	134	140	90	Nor.	N	—	Arrested
534	29	M	130	145	80	Nor.	N	—	Improved
535	25	M	130	140	85	Nor.	N	—	Arrested
536	18	F	128	128	80	Nor.	N	—	Stationary
537	35	M	150	150	72	Nor.	N	—	Improved
538	20	F	115	140	100	Nor.	N	—	Improved
539	25	M	135	135	80	Nor.	N	—	Improved
540	38	M	150	150	72	99.2	N	+	Improved
541	30	F	100	135	72	Nor.	N	—	App. Cured
542	33	F	100	135	90	Nor.	N	—	Arrested

## DR. HYDE'S CASES

Case No.	Age	Sex	Blood Pressure		Pulse Av.	Temp. F. Av.	Urine	Degree of Toxemia	Status on Discharge
			Ad.	Av.					
1	30	F	128	136	84	Nor.	N	—	Arrested
2	42	M	135	145	82	Nor.	N	—	Arrested
3	28	M	114	110	98	100.0	N	+	Stationary
4	27	M	132	134	78	Nor.	N	—	Arrested
5	28	M	120	128	77	Nor.	N	—	Arrested
6	51	M	106	115	86	Nor.	N	—	Improved
7	24	M	130	136	80	Nor.	N	—	Arrested
8	67	F	116	120	86	99.2	N	+	Improved
9	27	M	110	128	80	99.0	N	+	Arrested†††
10	35	F	118	130	74	Nor.	N	—	Arrested
11	34	F	136	130	70	Nor.	N	—	Arrested
12	26	F	120	124	90	99.0	N	+	Improved
13	39	M	138	140	58	Nor.	N	—	Arrested
14	24	M	122	136	76	99.2	N	+	Improved
15	26	M	112	138	84	Nor.	N	—	Arrested
16	23	F	128	126	90	99.0	N	+	Improved
17	26	M	114	132	84	Nor.	N	—	Arrested†††
18	30	M	122	130	54	Nor.	N	—	Arrested
19	27	F	124	132	80	Nor.	N	—	Arrested
20	25	F	126	134	82	Nor.	N	—	Arrested
21	45	F	145	152	76	Nor.	N	—	Arrested
22	26	M	125	140	80	99.0	N	+	Arrested
23	45	F	140	150	76	Nor.	N	—	Arrested
24	28	M	130	134	80	Nor.	N	—	Arrested
25	27	M	138	146	80	Nor.	N	—	Arrested
26	24	F	122	134	76	Nor.	N	—	Arrested
27	23	F	106	114	82	Nor.	N	—	Arrested§§§
28	29	M	122	132	80	Nor.	N	—	Arrested
29	30	F	122	134	76	Nor.	N	—	Arrested
30	28	F	118	130	82	99.2	N	+	Arrested
31	41	M	138	138	74	Nor.	N	—	Arrested
32	24	M	112	124	80	Nor.	N	—	Arrested
33	34	F	128	132	86	Nor.	N	—	Arrested
34	29	M	134	136	94	99.4	N	+	Stationary
35	27	M	140	150	72	Nor.	N	—	Arrested
36	24	M	114	130	70	Nor.	N	—	Arrested
37	29	M	126	134	68	Nor.	N	—	Arrested
38	30	M	120	128	74	Nor.	N	—	Arrested
39	27	M	145	140	74	Nor.	Alb.	—	Arrested
40	35	M	128	134	78	Nor.	N	—	Arrested
41	23	F	118	130	84	Nor.	N	—	Arrested
42	25	F	110	126	86	Nor.	N	—	Arrested
43	32	M	126	140	84	Nor.	N	—	Arrested
44	28	M	120	120	100	94.4	N	+	Stationary
45	31	M	110	134	90	Nor.	N	—	Improved
46	36	M	118	136	80	Nor.	N	—	Arrested
47	24	M	112	136	70	Nor.	N	—	Arrested
48	26	F	108	124	76	99.4	N	+	Arrested
49	28	M	117	132	90	100.0	N	+	Improved
50	30	M	110	120	55	Nor.	N	—	Arrested
51	30	F	120	132	88	Nor.	N	—	Improved
52	30	F	104	110	112	101.2	N	++	Improved
53	36	F	118	138	74	Nor.	N	—	Arrested
54	29	F	120	132	86	Nor.	N	—	Arrested
55	25	F	116	136	86	Nor.	N	—	Arrested
56	16	M	118	126	80	99.2	N	+	Improved
57	31	M	120	136	76	Nor.	N	—	Arrested
58	37	M	128	144	84	99.0	N	+	Improved

††† Tuberculosis of the intestines.

‡‡‡ Hemorrhage.

§§§ Mitral stenosis.

changed little from that day to this. But there existed no scientific fact for such belief. It remained for altitude workers of later years to determine the factors that favorably influenced the course of the disease. And in a careful study of these observations one is forced to the conclusion that altitude is the chief element in the question of climate. As proof of this statement we would cite you the excellent work of Williams<sup>14</sup> of London on the marked expansion of the thorax with greater amount of oxygen content in the blood; the work of Webb and Williams<sup>15</sup> of

TABLE 2.—BLOOD-PRESSURE DATA CONCERNING FIFTY MALES. NORMAL INDIVIDUALS

Case No.	Age	Blood Pressure	Case No.	Age	Blood Pressure
1	51	142	26	25	135
2	29	144	27	23	116
3	36	145	28	23	116
4	25	152	29	44	148
5	30	138	30	55	175
6	66	125	31	27	154
7	22	142	32	23	135
8	28	154	33	21	162
9	54	134	34	21	152
10	42	116	35	21	138
11	52	165	36	28	125
12	31	130	37	28	155
13	36	154	38	21	154
14	52	154	39	28	150
15	27	164	40	21	140
16	50	170	41	21	135
17	46	150	42	37	170
18	38	122	43	37	125
19	19	136	44	43	135
20	30	162	45	35	150
21	43	144	46	48	125
22	38	134	47	55	148
23	35	132	48	41	140
24	22	120	49	38	154
25	34	150	50	43	148

Colorado Springs showing a marked lymphocytosis in altitude; and the painstaking experiments of the English Commission,<sup>15</sup> working with American collaborators, showing over a 40 per cent. increase in erythrocytes and proving beyond doubt that the increased hemoglobin content of the blood was not relative but actual.

Now, granting with Emerson,<sup>11</sup> that the result of hypotension in tuberculosis, or in any other condition, is insufficient capillary pressure,

14. Williams: Trans. Internat. Cong. Tub., 1908.

15. Webb and Williams: Trans. Nat. Assn. for Study and Prev. Tub., 1909.

16. Douglas and Haldane, Schneider and Henderson: Physiological Experiments on Pike's Peak, Summer, 1910.



more or less venous stagnation, and insufficient nourishment with resulting atrophy and degeneration of the essential organs of the body, why then, we ask, if our work is confirmed by other observers, is this factor of increased blood-pressure at high altitude not an essential element in the treatment of all forms of tuberculosis? We feel from our own standpoint that we have shown conclusively that such an increase occurs and we now await with interest a confirmation or a refutation of our work by competent observers.

TABLE 3.—DATA REGARDING BLOOD-PRESSURE IN TREATED PATIENTS

Average blood-pressure on admission in 542 cases.....	124
Average blood-pressure during treatment in 542 cases....	132
Average increase .....	8
Number of cures .....	110
Average pressure on admission.....	124
Average pressure during treatment.....	135
Average increase .....	11
Number arrested .....	153
Average pressure on admission.....	125
Average pressure during treatment.....	137
Average increase .....	12
Number improved .....	171
Average pressure on admission.....	123
Average pressure during treatment.....	133
Average increase .....	10
Number stationary .....	36
Average pressure on admission.....	130
Average pressure during treatment.....	130
Average increase .....	0
NOTE.—The high average pressure here is due to two kidney cases with pressures of 164 and 148, which taken out, gives an admission average of 128 and a treatment average of 129, with increase of 1.	
Number of progressives.....	59
Average pressure on admission.....	122
Average pressure during treatment.....	115
Average decrease .....	7

We give here a table of six hundred cases (Table 1). We are indebted to Dr. O. T. Hyde of St. Joseph's Sanatorium, Silver City, N. M., for the Faught records of fifty cases (Table 2). To eliminate the personal equation we also append a table (Table 5) showing a comparison of the readings on a Stanton and an Erlanger. In the entire series of cases we have used at different times a Janeway, a Stanton and a Faught. In all readings the cuff was applied to the left arm above the elbow, the patient in a sitting posture, the forearm flexed on a line with the heart. The systolic pressure alone was recorded since for all practical purposes we feel that this is sufficient, and also, we do not believe that an accurate diastolic reading can be made with an ordinary

instrument. The observations were made between the hours of nine and ten in the morning as nearly as this was possible in our routine work. The blood-pressure is given on admission, as is also the average for the time under observation, this time being about seven months. In recording the pulse and temperature we have taken an average during the stay at the sanatorium. The degree of toxemia is represented as follows: — = a normal temperature; + = 99 to 100; ++ = 100 to 101; +++ = 101 and over. It is interesting to note that the averages of Dr. Hyde's cases taken independently of our own work shows an average on admission of but two points lower than our own and exactly the same average during treatment, and an analysis of his cases proves the points brought out by the analysis of our own table.

TABLE 4.—RELATION BETWEEN TOXEMIA AND BLOOD-PRESSURE

Number of cases with minus toxemia.....	300
Average pulse .....	85
Average blood-pressure .....	124
Number of cases with plus toxemia.....	184
Average pulse .....	72
Average blood-pressure .....	132
Number of cases with two plus toxemia.....	46
Average pulse .....	105
Average blood-pressure .....	120
Number of cases with three plus toxemia.....	11
Average pulse .....	115
Average blood-pressure .....	111

A detailed study of the facts noted in the general column shows some interesting relations which are grouped separately (Table 3).

From a study of the tables referred to we find that in all cases showing a betterment in pulmonary condition we get an increase in blood-pressure; in all cases showing no improvement we find the pressure practically the same; in all showing a progressive tendency we find a decrease in blood-pressure.

Classifying the minus and plus degree of toxemia as a class showing practically no toxic absorption, we find that the average pulse is 78, and the average pressure 128. Then further combining the two plus and three plus toxemia as a class showing marked toxic absorption, we find the average pulse to be 110 and the average blood-pressure to be 115. Here is shown the inverse ratio of pulse and blood-pressure, namely, the higher the pulse, the lower the blood-pressure, and *vice versa*. It also shows clearly that the greater the degree of toxemia the lower the blood-pressure, and the less the degree of toxemia the higher the blood-pressure.

As has been previously stated, pressures were taken with the Stanton and Erlanger apparatus during the same sitting in order to determine the comparative value of the cheaper instruments. We here append a table of forty cases showing this comparison (Table 5).

TABLE 5.—COMPARISON OF BLOOD-PRESSURES TAKEN WITH STANTON AND ERLANGER INSTRUMENTS

	Erlanger	Stanton
1 .....	115	114
2 .....	145	140
3 .....	130	124
4 .....	124	120
5 .....	128	124
6 .....	122	125
7 .....	148	146
8 .....	126	126
9 .....	128	128
10 .....	110	110
11 .....	114	114
12 .....	164	164
13 .....	134	134
14 .....	128	132
15 .....	136	135
16 .....	148	144
17 .....	130	124
18 .....	142	136
19 .....	138	138
20 .....	112	114
21 .....	126	126
22 .....	136	136
23 .....	134	136
24 .....	116	116
25 .....	132	130
26 .....	134	134
27 .....	120	122
28 .....	132	134
29 .....	124	124
30 .....	132	134
31 .....	124	124
32 .....	120	122
33 .....	116	116
34 .....	144	142
35 .....	138	138
36 .....	132	130
37 .....	110	110
38 .....	124	122
39 .....	128	126
40 .....	110	115

Average pressure, Erlanger, 129; Stanton, 128.

Table 5 would indicate that the readings of the cheaper instruments are practically the same as those of the Erlanger.

#### CONCLUSIONS

From a study of six hundred cases we draw the following conclusions:

1. The blood-pressure is increased at elevations of 6,000 feet.
2. The blood-pressure of both normal individuals and consumptives is higher at 6,000 feet than at sea level.
3. The pressure tends to increase up to certain limits with continued residence.
4. From a prognostic standpoint the blood-pressure findings are of great value in tuberculosis.
5. There is no relation between the degree of involvement and blood-pressure, but there is a constant relation between the degree of toxemia and blood-pressure.

## "AURICULAR FLUTTER," WITH A REPORT OF TWO CASES \*

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In 1911 Jolly and Ritchie<sup>1</sup> reported a case of Adams-Stokes disease which had been under observation for a period of six years and which showed in association with the complete heart-block a very rapidly beating auricle, the rate of the latter varying from time to time, but usually from 270 to 300 per minute. This condition they called auricular flutter. In adopting the term they refer to the work of McWilliams,<sup>2</sup> who, years ago, observed that the application of a faradic current started the auricle into a rapid flutter. In his account, McWilliams stated that the contractions originated in the stimulated area and extended rhythmically and coordinately throughout the tissue. In 1909, Hertz and Goodhart<sup>3</sup> reported a case with an auricular rate of 234 and a ventricular rate varying from 72 to 120. In this case the irregular and varying ventricular rate depended on a partial heart-block, although this was not recognized at the time. Lewis<sup>4</sup> has since written a very comprehensive article on the subject in which he reports eight cases which have come under his own observation and eight other cases which have appeared in the literature from time to time and which apparently are all types of this disorder. Ritchie<sup>5</sup> has also recently discussed the subject and reports four cases in which the condition has been definitely proved to be auricular flutter, and three others in which he believes that to have been the mechanism. These include the case reported in his original article. Hume's<sup>6</sup> case, no doubt, belongs in this category.

Auricular flutter when first described was considered a rare condition. As a matter of fact, it is probably comparatively common. The reason for its apparent rarity is that it is impossible to detect these extreme accelerations except by the use of graphic methods, and unless a routine examination by these methods is carried out many of the cases will escape recognition. The condition is an abnormal cardiac mechanism

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\* Read at the meeting of the American Climatological Association, Washington, D. C., May 6, 1913.

\* Submitted for publication July 8, 1913.

1. Jolly and Ritchie: *Heart*, 1911, ii, 177.

2. McWilliams: *Jour. Physicians*, 1887, viii, 296.

3. Hertz and Goodhart: *Quart. Jour. Med.*, 1909, ii, 213.

4. Lewis: *Heart*, 1912, iv, 171.

5. Ritchie: *Edinburg Med. Jour.*, 1912, ix, 485.

6. Hume: *Quart. Jour. Med.*, 1913, vi, 235.



characterized by a rapid, rhythmic, coordinate contraction of the auricle, the rate usually being somewhere between 200 and 300 per minute. A notable characteristic is the constancy in the auricular rate, any change due to posture or exercise being almost entirely negligible. Some degree of heart-block is usually present; consequently the ventricular rate is slower and may be regular or irregular.

It is a widely accepted belief that the normal cardiac impulse is brought about by a stimulus which arises in the sino-auricular node, sometimes called the pacemaker, situated near the mouth of the superior vena cava. This node gives rise to impulses in a healthy adult at the rate of about 72 to the minute. The rate is subject to wide variations as the result of exercise, emotion, fever and various other causes. The details of the mechanism which controls the rate are by no means clearly understood; whether the ordinary accelerations are due to diminished vagus tone or to direct stimulation through the sympathetic or to some other cause, it is not easy to prove, but, at any rate, it seems clear that all these simple accelerations are under the control of this mechanism whatever it is, and that all the impulses come from the normal point of origin. These have been called tachycardias of a physiological type, because they are simply the normal phenomenon exaggerated.

In auricular flutter the predominating evidence is that the origin of impulse is ectopic; that is to say, some other point than the normal pacemaker has taken on the function of stimulus production. It is well known that the ordinary premature auricular beat is the result of some pathological, usually ectopic, impulse formation. If a series of these beats arise in quick succession we recognize the condition clinically as paroxysmal tachycardia. This is usually characterized by a rate of 110 to 200 — rarely over 180. If, however, this abnormal focus in the auricle gives rise to impulses at a rate greater than 200, the condition has been called auricular flutter. This may seem an arbitrary division, for etiologically and pathologically it apparently does not differ from the simple paroxysmal type, but clinically it differs sufficiently so that it is considered advisable that it be classed in a separate category. It has been found, also, to have a close relation to auricular fibrillation, and when one considers the pathology and mechanism it is easy to see how readily a condition in which there is one abnormal point taking on the function of stimulus production might pass into a condition in which many foci have appropriated that function.

Some of the reasons for believing that the stimulus which gives rise to this action is ectopic in nature are as follows: 1. The already-mentioned close relation between this and the isolated premature auricular beat, the simple paroxysmal tachycardias and auricular fibrillation. 2. The auricular complex as recorded by the electrocardiograph

differs essentially from those which rise from the normal pacemaker. 3. The rhythm is not under nerve control as is the normal rhythm, being practically unaffected by posture, exercise or nerve stimulation.

The rate of the auricular beat varies considerably. In the cases collected by Lewis it has varied from 200 to 330 per minute. As already mentioned, this rate is remarkably constant, although observations made at long intervals of time show some variation in the rate. Observations made on the same day or on succeeding days usually show but little if any change.

The ventricular rate is usually slower, due to some degree of heart-block. The most frequent condition is a 2:1 ratio, in which the ventricle responds to each alternate auricular contraction. However, there may be any variation and instead of 2:1, the response may be at the ratio of 4:1. In either instance the ventricular rate is regular. If, however, as it is now known frequently occurs, the responses are mixed so that a 2:1, 3:1, 4:1, 5:1, etc., may be present, the pulse becomes entirely irregular and may be with difficulty distinguished from auricular fibrillation. The ratio of response may vary with exercise so that a patient who, lying down, would have a regular pulse at the ratio of 4:1, might, when standing, have a regular pulse with a 2:1 response, or a patient with a regular pulse with a ratio of 2:1 while active might have an irregular pulse because of mixed responses when at rest. In most of these cases the original grade of heart-block is increased by digitalis or strophanthus, which suggests that there is probably some impairment of muscle bundle conduction, for it is believed that these drugs have little, if any, influence on the conduction in a normal heart. Following the use of these drugs the regular 2:1 response may be converted into a condition of mixed responses of 2:1, 3:1, 4:1, 5:1. An interesting fact is that in the majority of instances these cases will pass from a 2:1 to a 4:1 ratio rather than from a 2:1 to a 3:1, and if the ventricle is irregular it is likely to consist of mixed 2:1 and 4:1 periods, though as Case 2 of those I am reporting shows, this is not always true. Isolated 3:1 or 5:1 periods may occur, but they are comparatively rare, and when a 3:1 ratio occurs successively the periods are usually short.

#### THE POLYGRAPHIC TRACINGS

The first case of Jolly and Ritchie, in which there was complete heart-block, and in which the jugular tracings were taken by a Knoll-Hering polygraph, showed distinctly the auricular waves. On the other hand, in many cases the jugular tracing is of no value in the diagnosis and may even be of such form as to be entirely misleading. In fact, the venous pulse in a 2:1 ratio is likely to be of the ventricular type. Occasionally when the ratio is 4:1 one may get an auricular wave toward the end of the ventricular pause and if, as sometimes happens, there is an

unusually prolonged pause there may be a series of auricular waves toward the end of it. Sometimes, as in one of my cases, the auricular waves are so prominent as to seriously complicate the c waves and in the attempt to identify the latter one may get a clue to the condition. The radial tracings are often of much more value in the analysis than the jugular and a close examination of the arterial pulse-curve alone may enable a positive diagnosis to be made. The points to be borne in mind as given in detail by Lewis are: 1. Alternation is commonly present. 2. The strength of the beats is substantially influenced by the preceding pauses. This is true, however, only when the pauses are too short for the ventricular contraction to be of maximal efficiency. 3. In the heart-block of flutter there is considerable variation in the As-Vs interval which modifies the expected pauses—a long pause is followed by a shortened conduction interval and a short pause by a lengthened conduction interval. 4. The weak beats are likely to be preceded by a relatively long presphygmic interval. All these factors must be considered, and contribute to the difficulties of the analysis. Usually when the ratio of ventricular response is irregular there is a definite grouping of beats, so that groups of three or four beats of irregular lengths may be repeated over and over again; but in some instances the ratio of response is so mixed that the condition is distinguished from fibrillation with difficulty.

#### TREATMENT

The general management of the case depends on the symptoms. If there is evidence of cardiac failure, of course, rest in bed is of paramount importance. The latter is just as beneficial in this condition as in any other in which the heart muscle is overtaxed. This was shown in Case 2 in which the patient had no medication for four days after admission to the hospital, but whose symptoms improved progressively from the time of admission though the pulse remained unchanged. Ordinary sedatives should be used as indicated in the individual case.

The particularly beneficial drugs, however, are digitalis and strophanthus. In several of the reported cases, during the administration of digitalis the flutter has passed into fibrillation. Then when digitalis is withheld the fibrillation has disappeared and the normal rhythm has become reestablished. Lewis explains this by saying that fibrillation seems to submerge the abnormal fast rhythm, which may not recur when the fibrillation passes off. As has already been stated, digitalis and strophanthus usually slow the heart by increasing the grade of heart-block so that these drugs may act beneficially in either of these two ways, namely, by causing fibrillation, which may subside, leaving the rhythm normal, or by slowing the pulse through increased heart-block.

In Case 2, digipuratum grs.  $1\frac{1}{2}$ , was given four times a day for eight days. At the end of that time, as indicated by the tracing taken on March

31, there was some increase in the grade of heart-block, causing a slowing of the pulse with some characteristic irregularity. What the dominant mechanism was during the next month is a matter of conjecture, but presumably flutter was persisting all this time, and on April 29 a tracing showed what appeared to be a 4:1 block. The amount of digitalis the patient had been having at that time is not definite. The patient was put on digitalis subsequent to this and a tracing taken on May 15 showed that the auricle had gone into fibrillation. The date on which this occurred is not known. Whether the fibrillation will be permanent or whether the normal rhythm will become reestablished, as frequently happens, can not at present be stated.

I shall report two cases, showing typical polygraphic tracings, which, when analyzed, leave no doubt that the mechanism present is that of auricular flutter. I have not been able to get electrocardiograms but have had to depend entirely on the polygraph.

#### CASE REPORTS

CASE 1.—Edwin C., aged 48, was admitted to the Rhode Island Hospital, Nov. 20, 1912, and came under my care Jan. 1, 1913. The patient is a native of Rhode Island and a jeweler by occupation. His family history is unimportant. He gives no history of any previous illness which might have any bearing on his present condition. He drinks considerable alcohol habitually, and occasionally to excess. A year before admission he began to get short of breath on exertion. About five weeks previous to my examination his legs began to swell and shortly before admission the swelling had extended to his hands, face and genitals. He had to get up at night to void urine occasionally. He was at work until within a week of his admission to the hospital. On admission there was slight swelling of all of the extremities and of the genitals, with some edema of the abdominal wall, but no definite signs of fluid in the abdomen. There were moist râles in the bases of both lungs behind. Heart apex was in the fifth space 8 cm. from the mid-line and the organ was not definitely enlarged. Sounds were regular and clear, the aortic second slightly accentuated. The pulse was regular, of good volume and tension. Examination of the urine showed a trace of albumin with a moderate number of hyaline and granular casts. The quantity was rather scanty. The functional test with phthalein showed 20 per cent. efficiency. Patient had a good deal of dyspnea which came on periodically, some days being much less marked than others. It was relieved with morphin. His condition remained the same for a number of weeks, except that the edema of the extremities was increasing and the dyspnea was perhaps more marked. His pulse was noted frequently and was always regular. At the time of the morning visit, on the morning of February 10, it was noted to be rapid and irregular. A tracing of the arterial pulse was taken at that time, portions of which are shown in Figures 1 to 4, inclusive. These tracings show considerable stretches of an absolutely regular pulse at the rate of 143 per minute. Interspersed here and there in the tracing are irregularities of greater or less length. The evidence afforded by analysis is that the auricle is beating at the rate of 286 per minute and that the regular strips are a 2:1 response of the ventricle, while the irregularities are markedly irregular, the ratio of 2:1, 3:1, 4:1, 5:1, all being present.

While the dyspnea did not seem any more distressing with the onset of this irregularity, the edema did increase more rapidly after this rhythm was established and was relieved only by the use of Southey's tubes. In a tracing taken February 20 (Fig. 5) the irregularity was quite different, at this time a very





definite grouping being quite persistent. For example, a group of five beats of varying lengths is repeated over and over again, the entire length of the group

being  $\frac{19.5}{5}$  seconds. The estimated ratio in these groups is 3 : 1, 5 : 1, 3 : 1.

4 : 1, 5 : 1, which would give an auricular rate on this day of about 308. Occasionally there is a break in this group.

This analysis of Figure 5 would seem most probably correct, though the evidence is not absolute.

The patient was given tincture of digitalis m. xv t.i.d. from February 20 to March 15. The irregularity continued until March 10, when it was noted at the morning visit that the pulse was regular. The tracing taken at that time showed the rhythm to be normal except for its modification from the Cheyne-Stokes breathing (Fig. 6). The patient is still in the hospital without any very material change in his condition. At times his dyspnea is marked and at times the Cheyne-Stokes breathing is marked, but there are other times when both of these are entirely absent. The edema is still quite considerable and although it cleared up materially with the return to the normal pulse rhythm, it is still necessary to remove some of it by Southey tube drainage.

#### SUMMARY OF CASE 1

A man of 48 suffering from progressive chronic nephritis with paroxysmal dyspnea and considerable edema, suddenly developed an irregular heart action after nearly three months in the hospital. This lasted for a month, when the rhythm suddenly became normal. There were no noticeable symptoms with the onset or end of the attack but the edema was considerably more marked while the condition persisted. Cheyne-Stokes breathing was present at the time of the return to the normal rhythm. Digitalis in moderate doses of the tincture was being administered during most of the time of the irregularity. The tracings indicate that the irregularity was due to a very rapidly beating auricle, associated with irregular ventricular responses.

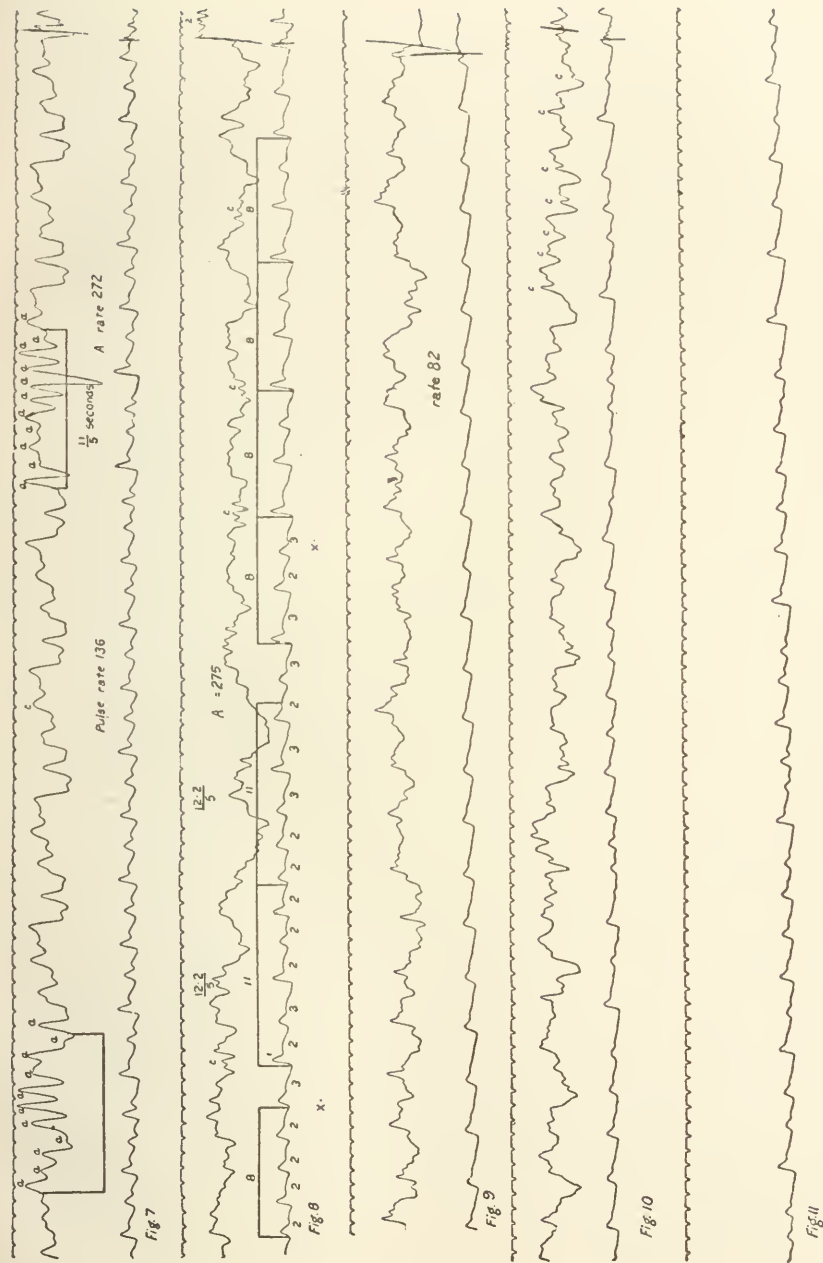
CASE 2.—W. K., aged 54, was admitted to my service at the Rhode Island Hospital, March 17, 1913. The patient was a native of Rhode Island, a jeweler by occupation and came in complaining of shortness of breath and swelling of the legs. The physician who attended him before admission said that for a month his dyspnea had been marked and that it was with difficulty that he could go up even a slight incline. The trouble had been coming on gradually for a year but in November, 1912, he had an attack of bronchitis with a persistent and troublesome cough which aggravated the condition, so that there was increased shortness of breath, some swelling of the legs and considerable dizziness on exertion. He had to get up at night five or six times to void urine and was troubled a good deal with constipation. His general health, he stated, had always been good although he had had hay-fever in the summer for a number of years. On admission there was a good deal of dyspnea with orthopnea, some signs of a slight amount of fluid in the right chest and râles, with a distinct pleural friction. The heart was slightly enlarged and action very rapid but regular, there were no murmurs. His blood-pressure while in the hospital varied from 160 to 190. There was marked edema of both legs, with some dermatitis. The amount of urine averaged from 900 to 1,200 c.c. with a specific gravity from 1.009 to 1.012 showing a slight trace of albumin but no casts. The heart action was so rapid as to attract attention at once and a polygraph tracing was made. It showed a rate of 138 per minute, with marked alternation of the pulse. Patient, at first, received no medication. Two days later another tracing was taken showing a pulse rate of 136 and still with

marked alternation. The venous tracing on this day showed in places auricular waves at the rate of 272 per minute (Fig. 7). March 22, four days after admission, a third tracing was taken, showing the pulse rate 137. The patient had been in bed during the four days he had been in the hospital, but was at this time allowed to get up and was given some exercises at the side of the bed and a tracing was taken immediately afterward. The rate was absolutely unchanged. March 23 the patient was given digipuratum  $1\frac{1}{2}$  grs. four times a day. This was continued until March 31. The pulse was observed repeatedly and was always found to be regular, with the rate practically unchanged. The first irregularity was noted March 31 after eight days treatment with digitalis. A tracing made on that day shows a very interesting condition (Fig. 8). There are short runs of beats of the same length as had been present during all the time when he was under observation, but between these runs are periods of irregularity. Considering the condition while the pulse was regular to have been a 2 : 1 heart-block as evidenced by the venous tracing, analysis of the irregular curve shows a heart-block of a 2 : 1 ratio in the regular strips and mixed 2 : 1 and 3 : 1 ratios in the irregular strips. The irregularity is usually made up of groups of three beats, one of which is at the ratio of 2 : 1, the following two beats at the ratio of 3 : 1. In other words, when the pulse is regular there are four ventricular beats for every eight auricular beats, while in the irregular periods there are three ventricular beats for every eight auricular beats. The patient left the hospital on March 31, the day on which this tracing was made. He was not seen again until the afternoon of April 29. He had gone to work and had been able to work for a week. His old symptoms, however, returned so that he had to give up. The edema on this last date was again quite marked. He was sitting up in a chair and had spent several nights sitting up, not being able to sleep. He was having slight attacks of paroxysmal dyspnea. Cheyne-Stokes breathing of moderate type was present. The patient was able to move about the house without much evidence of shortness of breath. A tracing was taken which showed a regular pulse, rate 82 to the minute with slight alternation (Fig. 9). The tracing was continued at intervals for about an hour but at no time was there any irregularity whatever. The rate was absolutely constant. The patient twice got up from the chair, walked about the room and a tracing taken immediately after showed no change in the rate. The jugular tracing was not very satisfactory and while there were some extra small waves, there was nothing characteristic enough to make it absolutely sure whether the flutter is still persisting or not. The absolutely persistent rate unaffected by exercise suggests very strongly that the condition was still present and that there was a 4 : 1 block. Urinalysis: sp. gr. 1.020, trace of albumin, with a number of small hyaline casts. The patient was again seen May 15, when a tracing showed that the auricle had passed into fibrillation (Figs. 10 and 11).

#### SUMMARY OF CASE 2

A man aged 54, with gradually increasing dyspnea and edema for a year, comes under observation with chronic nephritis and an enlarged heart in stage of decompensation, and a pulse of 136 to 138 uninfluenced by posture or exercise. Auricular waves are present in the venous tracing at the rate of 272 per minute, indicating a 2:1 block. With rest in bed improvement was steady but the pulse was not slowed until after eight days of digitalis, when it became irregular, the latter being due to an increased grade of heart-block. Six weeks later the auricle was found to be in fibrillation.

Ritchie recognizes four groups of cases, the division being based on the symptomatology. These groups are as follows:



TRACINGS IN CASE 2.—Figure 7, two days after admission. The pulse is for the most part regular at a ratio of 2 : 1. At two points it suggests a 1 : 1 and 3 : 1 ratio. The auricular rate is 272. Alternation of the pulse is well marked. Figure 8, after eight days of digitalis, showing mixed responses at 2 : 1 and 3 : 1 ratio, with short runs of 2 : 1. Patient left the hospital and was not observed for a month. Figure 9, tracing made April 29, one month later. The pulse was regular, 82 to the minute, unaffected by posture and exercise. Figures 10 and 11, taken May 15, show fibrillation.



1. Those in which there may or may not be signs of cardiac disorder but in which the symptoms are due to the rapid auricular and consequent rapid ventricular rate. It is perhaps in the cases of this group that the diagnosis of the condition is most difficult and also that it is of the most importance. For if there is no serious cardiac lesion and the auricles can be made to revert to their normal rhythm, the symptoms will quickly disappear and the patient may remain well for an indefinite period. Certain of the cases of this group, except for the greater rapidity of the auricular action, are hardly to be distinguished from ordinary paroxysmal tachycardia. The ratio of heart-block is likely to be 2:1 and unless some variation of this ratio occurs producing some irregularity, it may be impossible to arrive at a diagnosis from an analysis of the venous and arterial tracings. Persistent searching, however, is likely to discover some irregularity which may at once establish the diagnosis.

2. Cases of old standing cardiac disease in which the auricular tachycardia is apparently an incident in the progress of the disease. With the onset of flutter in these cases the condition usually becomes worse, as frequently happens with the onset of fibrillation.

3. Cases in which there is partial heart-block as a consequence of which the ventricular pulse is slow and likely to be irregular.

4. Auricular flutter associated with complete heart-block. In the latter, of course, the ventricular action is entirely unaffected by the rapid auricular rate.

It would seem reasonable to class the last two groups as one, inasmuch as the chief difference seems to depend on the grade of the impairment of conduction between the auricles and ventricles.

The two cases reported present certain points of similarity and would apparently fall into the second group mentioned by Ritchie. Both were near the end of the fifth decade of life. In each case there was some arterial hypertension, some chronic disease of the kidneys, some cardiac hypertrophy with cardiac insufficiency associated with dyspnea and edema. In one instance the onset of the abnormal rhythm and the reestablishment of the normal rhythm occurred while the patient was under treatment in the hospital. Tracings were not taken immediately before the rhythm returned to normal, so whether it passed through the stage of fibrillation is uncertain. The duration of the abnormal rhythm was about a month. In the other case, the auricle was in flutter when the patient came under observation. It passed into fibrillation about six weeks later and has remained in that state.

# The Archives of Internal Medicine

Vol. XII

NOVEMBER, 1913

No. 5

## THE CAUSE OF THE SPECIFIC DYNAMIC ACTION OF PROTEIN \*

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NEW YORK

On the occasion of the International Congress of Hygiene and Demography held in Washington in 1912, it was pointed out that the ingestion of 25 grams of glycocoll or of 20 grams of alanin, each of which is convertible in the organism into 20 grams of glucose, caused a very great rise in the quantity of heat produced by a dog, whereas the ingestion of 20 grams of glucose itself had a slight or a negligible influence. It was also shown that when glutamic acid was administered there was no increase in the heat production of the dog. Since glutamic acid yields both sugar and urea in the course of its metabolism, it was concluded that the increased heat production after giving glycocoll and alanin could not be due to the processes of sugar formation, of deamination or of formation or elimination of urea. Also, ingestion of urea gave negative results as regards increased heat production. It seemed, therefore, that the stimulus to greater heat production given by glycocoll and alanin must have been due to their own chemical individuality. It was further shown that leucin and tyrosin increased metabolism, and that a mixture of 5.5 grams of each of the five amino-acids, glycocoll, alanin, glutamic acid, leucin and tyrosin caused an effect on metabolism which was as pronounced as that observed after giving 25 grams of glycocoll alone. It was greater than that following the ingestion of 100 grams of meat which contained about the same quantity of nitrogen. That the effect of this mixture of the various amino-acids was one of summation is shown in the following new experiments.

The experiments were performed on Dog 3, weighing 12 kilograms, whose basal metabolism (that obtained eighteen hours after administering a standard maintenance diet) as determined on February 26, 27

\* Address delivered at the International Physiological Congress, Groningen, September, 1913.

\* From the Physiological Laboratory of the Cornell University Medical College.

\* Submitted for publication July 21, 1913.

and March 28, was found to average 19.8, 19.7 and 19.9 calories per hour. The amino-acids were given eighteen hours after the ingestion of food and the metabolism determined for three or four hours afterward. It was found in this way that the increase in heat production after giving 5.5 grams of glycocoll was 3.1 calories, after 5.5 grams of alanin it was 3.9 calories and after a mixture of 5.5 grams of each 8.5 calories. In other words, the basal metabolism was raised by glycocoll in this small amount 5.2 per cent., by alanin 6.6 per cent. and by the two mixed together 14.1 per cent. These experiments prove that there is a summation in the heat-increasing power when the two amino-acids are ingested together. This one would expect to find from the behavior of metabolism following meat ingestion.

The results of these experiments reinforced the conclusion already published that the increase in metabolism after the ingestion of meat is due to the mass action of absorbed amino-acids acting on cellular protoplasm.

An *experimentum crucis* was devised to determine whether glycocoll itself is a stimulus to metabolism. When glycocoll is administered in phlorhizin glycosuria its energy content is almost entirely eliminated in the form of sugar in the urine. It may be computed that 10 grams of glycocoll containing 31.10 calories of energy are converted into 8 grams of sugar with 29.52 calories and 4 grams of urea with 10.11 calories. The reaction is, therefore, endothermic. If the heat production be increased after giving glycocoll in phlorhizin glycosuria it would be certain that this would be due to the chemical nature of the substance and not to its energy content.

Considerable difficulty was occasioned by the fact that the fasting diabetic animal, when placed in the respiration calorimeter at a temperature of 26, almost invariably vomited the solution of glycocoll administered. After six experiments on four different dogs success was obtained. The record of metabolism began an hour and three quarters after the ingestion of 12.5 grams of glycocoll. As on other occasions, the dog manifested polypnea about an hour after administration of the glycocoll. On this occasion the animal was held in an upright position for over an hour before placing in the calorimeter, a procedure which often prevents vomiting. That the cause of the polypnea is due to the increased production of heat may be deduced from experiments of Wiggers, who introduced 4 c.c. of N/10 solution of glycocoll into the vein of an anesthetized cat without changing the nature of the respiration.

The *experimentum crucis* showed that the effect of glycocoll was to increase the fasting phlorhizin metabolism of 25.9 calories per hour to one of 31.4 calories, a rise of 21 per cent. A calculation from the urinary findings showed that within the short period of four hours an elimination

of 7 of the 10 grams of extra sugar which can arise from 12.5 grams of glycocoll, actually occurred. From former experiments there can be no doubt that essentially all the glycocoll was converted into sugar. A control experiment showed that the ingestion of 10 grams of glucose did not cause an increase in heat production, although 4.7 grams of extra glucose were eliminated in the urine during the period of three hours. Since the potential energy of 12.5 grams of glycocoll is largely eliminated in 10 grams of urinary sugar, and the actual elimination of sugar is not accompanied by increased heat production, it follows that the rise in metabolism after glycocoll ingestion is not derived from the energy content of the glycocoll administered, but that glycocoll itself acts directly as a stimulus to metabolism.

There is only one other possibility, namely, that advanced by F. G. Benedict, to the effect that organic acids are the effective stimuli. The chief support of Benedict's hypothesis lies in his statement that d-fructose increases the heat production in diabetics even though much of this sugar be converted into glucose, and none of it is oxidized. Here one might surmise that such intermediary products of sugar metabolism as lactic acid or methyl glyoxal (Dakin) might be the effective stimuli to increased heat production. In like manner, the oxy-acids glycollic or lactic, derived from glycocoll or alanin, or the keto-acids of the same series might be the effective stimuli after the ingestion of the amino-acids.

To investigate this problem, ethyl glycolate and ethyl lactate have been administered to dogs. When the former was given in doses of 12 grams it proved to be a toxic agent. Ethyl lactate, however, increased metabolism, and to a much higher extent than did its alcohol component given alone. Lactic acid itself has not been given, and experiments with glycollic acid have proved futile since it was vomited whenever administered. The work with ethyl lactate does not necessarily prove that lactic acid is in itself a stimulus to metabolism, as the effect produced might have been due to the action of some of the absorbed ester. The affirmation of Benedict's theory cannot, therefore, be given.

Pending further investigation it is well to recall that glutamic acid, which is a dicarboxyl amino-acid, exerts no influence whatever on heat production, although this substance forms urea and is in part synthesized into sugar in the body. This behavior favors the validity of the statement made a year ago that the increase in metabolism which follows the ingestion of meat is due to the mass action of amino-acids acting on cellular protoplasm.

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## SOURCES OF ERROR IN THE USE OF THE STOMACH-TUBE FOR DIAGNOSIS. PRELIMINARY REPORT \*

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It has been our experience that the unaided stomach-tube occasionally fails to detect gastric stasis which is demonstrable with the bismuth meal and Roentgen ray. Discrepancies in the Roentgen ray and stomach-tube findings were apparently not due to the position of the patient at the time of the passage of the tube; for we have observed them with patients in the sitting, supine and right reclining positions. The long axis of the stomach is more perpendicular than horizontal, but inclines somewhat to the right. Theoretically then, when the patient is reclining slightly on the right side, the gastric contents should occupy a narrower portion of the stomach than when the patient is in the erect sitting position, which is commonly employed in using the stomach-tube. The contents of the stomach in the slightly reclining right-sided posture should be of greater depth than when more spread over the greater curvature, as is the case in the erect sitting posture, and should, therefore, be more readily siphoned off with the tube. Even with the patient in this position we have failed with the tube to detect residuum which was demonstrable with the Roentgen ray. Curious to determine the cause of this inefficiency of the stomach-tube, we were led to watch by means of the fluoroscope, the course taken by the tube. We have observed in several individuals that the end of the tube did not pass directly to the most dependent portion of the stomach. In these cases, the tip of the tube first touched a portion of the stomach wall well above the most dependent portion. As more tube was passed, the end of the tube slid along the wall of the stomach a greater or lesser distance and became impinged against the stomach wall. On further passage of the tube, the end remained fixed and a downward bowing of the tube into the lower portion of the stomach occurred. This downward bowing of the tube apparently diverted the direction of force on the tip of the tube; for, as more tube was passed and the dependent loop of tube increased in size, the tip sooner or later slipped from its fixed position. The force on the tip was then upward through the dependent loop of tube and consequently the tip slipped upward toward the cardia; that is, it became further removed from any residuum which may have been present in the stomach. On passing still more tube, the end may follow about the wall of the stomach

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\* Submitted for publication, Aug. 18, 1913.

and may eventually reach its most dependent portion. We have observed, however, that it may again curve horizontally and then upward. The tip of the tube may skim the surface of the residuum and therefore drain only a portion of it. Further passage of the tube in the hope of reaching a deeper level of the contents may fail, because the tip may curve upward. The result in such cases may not be seriously deceptive. The degree of



Figure 1

stasis may be underestimated, but the analysis of the siphoned contents may show stasis. In some instances, however, considerable amounts of residuum may entirely escape detection by the tube. Although the accompanying skiagraphs do not show the course of the tube as satisfactorily as the fluoroscope, they are sufficiently indicative and require but little explanation.

Figures 1, 2 and 3 illustrate a successful passage of a stomach-tube. Figure 1 shows the outline of the stomach with bismuth meal. The lower border of the stomach is on a level with the inferior surface of the fourth lumbar vertebra. The patient is in a marked inclined position. In Figure 2, measuring from the incisor teeth,  $17\frac{1}{2}$  inches of the tube



Figure 2

have been inserted. The end of the tube is shown opposite the lower surface of the first lumbar vertebra and the bismuth residue opposite the middle of the fourth lumbar vertebra (just below the umbilicus, as indicated by the circular marker). Further passage of the tube was observed on the fluoroscopic screen and when the tip of the tube reached the bismuth residue, Figure 3 was taken. Twenty-six and one-half

inches of tube had then been passed, or  $3\frac{1}{2}$  inches beyond the tooth-mark on this tube.

Figures 4 and 5 illustrate the course of the tube in another case, an unsuccessful passage. The pictures were taken immediately after the



Figure 3



ingestion of a bismuth meal with a considerable amount of fluid. The patient is standing. There is no gastropnoxis, the lower border of the stomach being juat above the umbilicus, as indicated by the marker. As the tip of tube emerged from the esophagus into the stomach, the fluoroscope showed that it curved almost immediately to the left and became impinged against the stomach wall. On the passage of more tube a dependent loop formed. When 23 inches of tube had been passed, Figure



Figure 4

4 was taken. The fluoroscopic observations in the meantime showed the tip had remained practically stationary. The figure shows the tip of the tube an inch above the level of the fluid in the stomach. Even the lowest portion of the tube (the bottom of the dependent loop) is only at the top of the bismuth meal, that is,  $2\frac{1}{2}$  inches above the most dependent portion of the stomach. In Figure 5, 16 inches more of the tube have

been inserted, a total of 39 inches. It is seen, however, that the tip of the tube occupies practically the same position as in the previous figure. The tip is apparently firmly impinged. The dependent loop has increased in size and has advanced into the pyloric end of the stomach. Owing to the narrowness of this part of the stomach, the curve of the loop has



Figure 5

become sharper. The formation of this loop, as seen on the fluoroscopic screen, is shown in the accompanying diagrams (Fig. 6). Diagram (a) corresponds to Figure 4. Diagram (g) corresponds to Figure 5. Up to this point, the passage of 39 inches of tube, no fluid was siphoned from the stomach. The explanation is obvious from the figures, which show

that the tip of the tube was constantly above the level of the fluid. The tube was then gradually withdrawn, but still no fluid was siphoned off. The tip at no time receded beneath the level of the fluid, merely the loop was withdrawn.

Figures 7, 8 and 9 illustrate another unsuccessful passage of a stomach-tube. Figure 7 shows the size, shape and position of the stomach, the lower border being just below the umbilicus, as indicated by the marker, or on a level with the top of the fourth lumbar vertebra. Fluoroscopic observations revealed a very similar condition, as regards the relation of the tip of the tube to the cardia, as in the previous case. As the tube emerged from the esophagus, it curved directly toward the left and upward until it impinged against the stomach wall. As more

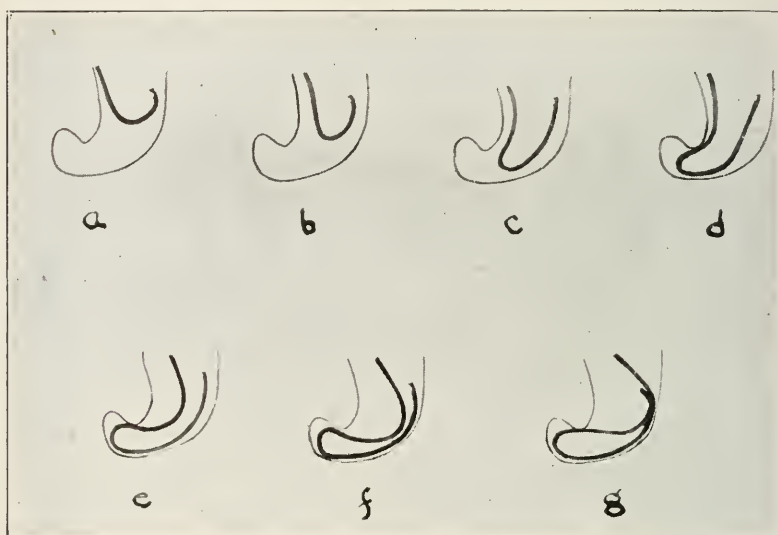


Figure 6

tube was passed a dependent loop formed. The tip finally slipped from its position against the stomach wall and came to lie at a higher level in the cardia. Figure 8 was taken when 35 inches of tube had been passed. The tip is in the *magenblase*, as indicated by the light space in the figure. The lowest part of the loop is just below the top of the third lumbar vertebra, or about 1 inch above the lowest portion of the stomach (see Fig. 7). In an effort to reach the lowest portion of the stomach with the tip of the tube, more and more tube was passed and constantly observed on the fluoroscopic screen. When 46 inches tube had been inserted, Figure 9 was taken. At no time during these fluoroscopic observations did the tip or any other part of the tube reach the lowest portion of the stomach. The figure shows the lowest portion of the tube

at the level of the middle of the third lumbar vertebra or about 1 inch above the lowest border of the stomach (see Fig. 7). The tip of the tube is on a level with the top of the second lumbar vertebra. The accompanying diagrams (Fig. 10) illustrate the course of the tube during its com-



Figure 7

plete passage as shown on the screen. Diagram (e) corresponds to Figure 8. Diagram (g) corresponds to Figure 9. It will be seen that the dependent loop of tube, in this case, descended into the lower portion



of the stomach and that the passage of more tube caused the formation of several kinks. At no time did the tip reach the lowest portion of the stomach, and, furthermore, the kinks themselves would have prevented the siphonage of any residue.



Figure 8

Curious to ascertain why the tip of the tube may become deflected in the cardia on emerging from the esophagus instead of passing directly to the most dependent portion of the stomach, we were led to pass stomach-tubes through a section of glass tubing about the length and diameter of the esophagus. We have frequently seen the tip of the stomach-tube, after emerging from the lower end of the glass, deflect

from the long axis of the glass. Stomach-tubes are delivered from the manufacturers coiled. Stomach-tubes are commonly kept coiled. Just before passing they are commonly coiled in basins or pitchers of ice or cold water. The tube acquires a tendency to curve, and we believe it is



Figure 9

the tendency so acquired which is responsible for its deviation from a straight course after emerging from the lower end of the esophagus. If the curve of the tube accommodates itself to the curves of the stomach wall and if the tip does not become impinged against the stomach wall,

the tip will reach the most dependent portion of the stomach. Deductions will be correct. If, however, the curve of the tube does not accommodate itself to the curves of the stomach wall and the tip becomes impinged against the stomach wall, the tip may not reach the most dependent portion of the stomach. Deductions will be incorrect.

Believing that it might be possible to allow for the curving tendency of the tube, we have started to pass the tube in such a position that, when it emerged from the lower end of a glass tube representing the esophagus, its tip should point toward the pylorus. This reasoning was shown to be fallacious; for the direction of the tip at times as it emerged from the lower end of the glass was contrary to expectations. The tube, in passing,

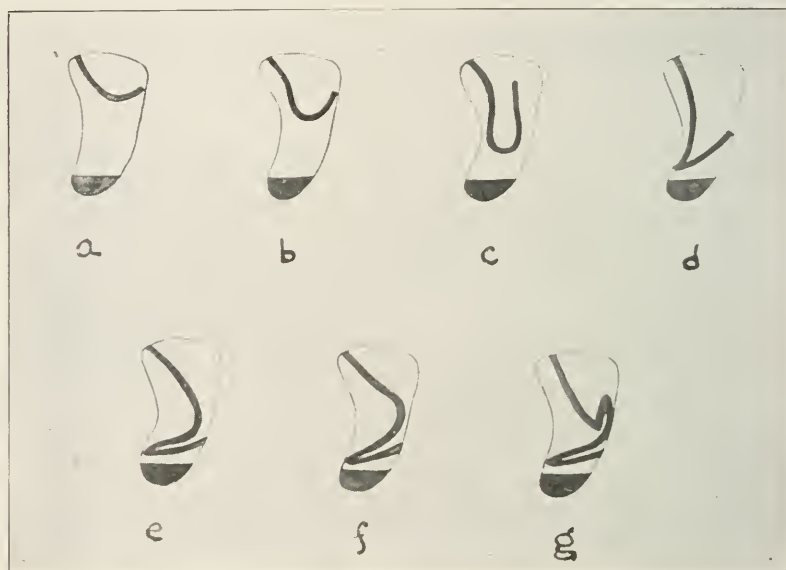


Figure 10

at times became revolved by its contact with the glass tubing, and this revolution could not be prevented with certainty.

The proportion of successful and unsuccessful passages of a stomach tube is dependent in part on the tightness in which the tube is kept coiled and on the flexibility of the tube. The more flexible is the tube, the less apt is it to maintain a curve after coiling, but the more apt is it to kink when its tip impinges against the stomach wall. The stiffer is the tube, the more apt is it to maintain a curve after coiling, but the more apt is its tip to slip along the stomach wall.

The proportion of successes and failures is also dependent on the posture of the patient and on the position the stomach occupies in the abdominal cavity. In cases of gastropotosis the degree of displacement

may be underestimated and an insufficient length of tube passed. This, we believe, is a common error. Contrary to our expectations, however, we have found in some cases of even marked ptosis that, if enough tube be passed, the most dependent portion of the stomach may be reached



Figure 11

despite the tendency of the tube to curve. In such cases it appears that a long, tubular cardia tends to guide the tube to the bottom of the stomach. Figures 11, 12 and 13 illustrate such a case.

Figure 11 was taken immediately after the ingestion of a full bismuth meal and shows the lower border of a long, tubular stomach 1 inch above



the symphysis pubis. In Figure 12, 23 inches of tube (that is, to the tooth-mark) have been passed. The tube is shown inclining toward the left border of the stomach, the tip opposite the top of the fourth lumbar



Figure 12

vertebra. During the passage of this tube, as in previous cases, fluoroscopic observations were constantly made and the tip of the tube was seen to enter the bismuth meal. Figure 13 was taken when 34 inches of tube had been inserted. The tip has emerged from the bismuth residue



Figure 13

and is on a level with the middle of the fourth lumbar vertebra. This case illustrates a successful passage of a tube and apparently the long, tubular form of the cardia and perhaps the conformity of the false pelvis tended to direct the tip to the most dependent portion of the stomach. In this case the passage of 31 inches of tube was necessary to reach the

lowest part of the stomach (that is, 8 inches beyond the tooth-mark on the tube used). Several facts were shown in this case. If exactly 31 inches of tube had been inserted, the bottom of the fluid would have been reached, siphonage would have been complete and deductions as to the amount of residuum correct. But as there is no way of knowing exactly when the lowest level of the residuum is reached, it is obvious that too much or too little tube may be passed. In this case, if somewhat less than 31 inches of tube had been passed, siphonage might have been established, but the passage of more tube on the cessation of flow, in the hope of reaching the lowest level of the residue, might have failed, owing to the passage of too much tube, the tip emerging above the fluid level. Under these conditions, the gradual withdrawal of tube in the hope of resubmerging the tip would not reestablish siphonage once broken. This fact may be easily demonstrated with a tube and a partially filled flask.

Further observations are being made on various types of stomach and experiments in the construction of a tube which will obviate these difficulties.

From these simple observations it is obvious that failure to recover gastric residuum with the unaided stomach tube from a fasting stomach or after the ingestion of a test meal cannot be accepted as conclusive evidence of the absence of gastric stasis.

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## THROMBOSIS \*

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In the year 1911, at the meeting of the *Deutsche Naturforscher und Aerzte*, I submitted a review on the subject of Thrombosis. The question was brought up because the increasing number of operations in surgical and gynecological practice has made a fatal embolism of the lung, the sequel of a thrombosis, of more and more frequent occurrence.

The more we try to obviate this dangerous complication, the more urgent is our obligation to find out the cause of thrombosis. Now, there is no doubt at all, to use a mathematical figure of speech, that thrombosis is the function of a number of variables. There is not a *single* cause, but quite a number of different conditions which are closely related to the occurrence of thrombosis. Among these may be mentioned here, first, changes in the blood-plasma (diminished or increased coagulability); secondly, changes in the blood elements (increased or diminished powers of agglutination); thirdly, changes in the blood flow (slowing and formation of eddies), and lastly, changes in the vessel wall itself (endothelial damage). An inquiry into the mechanism of thrombosis shows that sometimes one factor, sometimes another, plays the principal rôle.

The view that increased coagulability of the blood is an essential point for the production of thrombosis, has been strongly upheld, especially by clinical observers. The existence of this increased coagulability, and the likelihood that it is a promoting factor, or, better, an accompanying phenomenon of thrombosis, cannot be denied. But all histological research since the early work of Zahn, Eberth and Schimmelbusch, Welch and others, speaks for the view that in human beings the occurrence of fibrin coagulation is not the first stage of thrombosis, but that important changes in the morphological blood constituents precede it. These latter changes must be explained before the mechanism of thrombosis can be understood.

It is concerning the morphological structure of a thrombus, in continuation and amplification of my review mentioned above, that I wish to speak.

### MORPHOLOGICAL STRUCTURE OF THROMBI

Since we have known from the work of Zahn, on the one hand, and of Eberth and Schimmelbusch on the other, that the blood-platelets and leukocytes really have to do with the building of a thrombus, the idea

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\* The Cartwright Lectures for the year 1913, of the Association of the Alumni of the College of Physicians and Surgeons, Columbia University.



readily suggested itself that the whiter color of one part of a thrombus was to be referred to an accumulation of these elements, which would thus appear to constitute the first material laid down in the process. We have become accustomed to speak of white, mixed and red thrombi, but the relationship of these different appearances to one another, in spite of the early work of Zahn and others, has not been wholly and correctly appreciated in the literature of the subject. We must remember with Welch—to whom we must give thanks, as well as to the previously mentioned authors, for the most exhaustive experimental work on thrombosis—that in a completely finished thrombus, for example in the femoral vein, the variations in the colors are perfectly regular. The first part (*Kopfteil*) of the thrombus is chiefly of a white color, and represents the so-called white thrombus, and on this a middle part (*Halsteil*), mixed in color, and a deep red, distal and final portion (*Schwanzteil*) are subsequently laid down. The first part (*Kopfteil*) may be of the smallest possible size and extent; whereas the final red part (*Schwanzteil*) often forms the bulk of the thrombus, and may measure many centimeters. Thrombi in other parts of the venous system are built up on exactly the same principle.

It is easy to understand that red thrombi occur only where a white thrombus has more or less completely obstructed a vessel, and that they cannot occur where the white thrombus only partially obstructs the lumen.

Very long white thrombi can arise only when the blood-stream continues to pass through a vessel. From all these points a rule can be formulated which holds for the majority of all autochthonous thrombi in human beings; namely, that the white thrombus is the determining and peculiar factor in the whole process, and that red thrombi are only, so to say, accidental—they may occur but not necessarily. An exception would of course be the case of a pure red thrombus arising by autochthonous thrombosis, and not consisting of a piece of an ordinary thrombus which had been broken off and carried away. Such an event, if it occurs at all, is certainly very rare.

Our interest, then, centers on the question of what this *Kopfteil*, or first part of a thrombus, looks like under the microscope. The structure of it will perhaps give us an understanding of its mode of origin.

First of all, we must remember that the much quoted Zahn's markings on the outer surface of thrombi—the small ripple-like, net-like and linear markings—are only to be seen on the *Kopf* and *Halsteil* of thrombi, whereas in the red part of the thrombus they fade and soon totally disappear. These markings are merely fine, white elevated lines or ridges which are specially well brought out when the furrows between are reddish in color, or where a mixed red and white thrombus, as in the *Halsteil*, is present. Along with the explanation of this marking stands or falls the

whole problem of thrombus formation, so far as consideration of the majority of cases of autochthonous thrombosis goes. This decision appears at first sight a bold one, but will readily be understood when the microscopic structure has been taken into account. A longitudinal section through the *Kopf* and *Halsteil* of a thrombus shows that the very delicate surface elevation is only the summit of a framework of beams, which in delicate rings like a mass of coral, forms the skeleton of the whole thrombus. When a thrombus at quite an early stage in its development is examined, this framework is seen to be a finely granular mass which consists simply of an accumulation of blood-platelets. All the beams of this framework are surrounded by a border of polymorphonuclear leukocytes, by means of which they are differentiated even more sharply from the red blood-mass which fills up the numerous spaces between.

But it is not only the regular and definite separation of the different blood-elements which is surprising, but the further fact that all the beams of the framework stand in a very special relationship to one another. The beams follow one another at fairly regular intervals, and build up group-like systems, inside of which the direction of the beams is much the same. In addition, however, secondary beams are seen in these groups extending either upwards or downwards from the primary beam of the framework. It must be admitted that dissimilarities also occur here. The more we approach the pointed outer extremity of the *Kopfteil* the broader become the beams of the framework, the furrows between disappear, until finally the beams unite into a single mass, to form the pure white outer surface of the *Kopfteil*.

The most important point, however, in the structure of this whole system is that fibrin practically does not appear. When it is found, however, the strictest regularity governs its situation. The threads of fibrin shoot out first of all along the borders of the framework of blood-platelets just where the red blood touches the framework. Subsequently it penetrates more and more into the blood itself. A structure such as has just been described is the only sure token by which we can recognize microscopically the intravital origin of a thrombus.

With this explanation of this structure, we are enabled at the same time to understand the way in which a thrombus is built up. The explanation involves the solution of a complicated physical problem, which I can present here only in a general way, since, in spite of many discussions with my colleagues in the department of physics, I have been unable to get to the bottom of it. What the vital question is, we can state nowadays with certainty. This question is: Does the thrombus arise in the flowing or in the stationary blood-stream? So long as the view was held that white thrombi were built up from leukocytes, as Zahn pointed out, it had to be assumed that it was only in the flowing blood

that these thrombotic masses could be laid down. Zahn himself laid great stress on this point. But when it became practically certain that a thrombus takes origin exclusively from blood-platelets, uncertainty again arose, since the origin and meaning of these platelets was veiled in obscurity. While some observers affirmed the independence of these structures, many more considered them to be merely distintegration products of white and especially of red corpuscles. If the latter theory were true, a thrombus could quite well be considered to be formed from a small mass of dead and disintegrated red blood-cells, which had died because of the stopping of the blood-stream. But it could not explain why the heap of particles at once proceed to build themselves up into such a beautiful framework and divide themselves off so sharply from the red corpuscles.

Taking it for granted that the origin of blood-platelets is not from leukocytes or erythrocytes, but that they are independent elements existing by themselves in the blood-streams (as indeed the excellent researches of Deetjen have already suggested), it becomes quite clear to us that such accumulations of blood-platelets as occur in a thrombus can be deposited only when the blood is circulating. The important question of the origin of the blood-platelets can, in my opinion, now be regarded as settled. They take origin neither from white nor red corpuscles, but are, for so long as they circulate in the blood, independent structures. J. H. Wright of Boston was the first to point out their origin from the giant cells of the bone-marrow and spleen. In his important researches he was able completely to confirm the statement of Schridde that the megakaryocytes have a finely granular protoplasm except for a zone at the margin, which remains clear. He then showed that from the giant cells small portions are nipped or budded off in the form of small platelets, each of which consists of a finely granular central portion surrounded by a clear marginal zone. These are in fact the blood-platelets, and the granular center, the origin of which is thus readily understood, is identical with the structure often described, especially by Deetjen, as a nuclear structure in the platelet. That independent movement is possessed by these structures had already been shown by Deetjen. Wright's findings, doubted on many sides, were reinvestigated by Ogata in the Pathological Institute in Freiburg, and were all completely confirmed. In fact in fresh, warm material taken from different animals for investigation the budding off of platelets from the megakaryocytes can be plainly followed. At the time of the budding-off process the protoplasm of the giant cell is seen to have invaginated itself into a capillary in the form of a pseudopod; and thus the occurrence of the platelets in the blood-stream comes about.

The independence of the blood-platelets in the circulation can be proved in another and very positive way. If they arose simply by the disintegration of red corpuscles, then the destruction of a stationary column of blood, as can readily be produced in a doubly ligated vessel by the corrosive action of a small piece of silver nitrate, would be followed by a blood-platelet thrombus-building, just as occurs in the flowing blood-stream where a typical thrombus forms on a damaged piece of vessel wall. This question was investigated by Derewenko<sup>1</sup> in the Pathological Institute in Freiburg with striking results. He found not a trace of heaping-up of platelets in the doubly ligated vessel in spite of all the damage, whereas in a vessel through which the blood still flowed a typical thrombus had been built up from blood-platelets, showing the characteristic framework we have learned to recognize in the human subject.

We have concluded then, that the thrombus arising from the blood-platelets has its origin in the circulating blood-stream, and the question yet to be answered is: Why do the platelets build themselves into this peculiar framework? To this important question I can give no final answer. A few interesting points only may be raised. By the making of serial sections of human thrombi, and subsequently building up models, Ferge,<sup>2</sup> working in the Pathological Institute in Freiburg, was able to determine with certainty that the platelets do not form beams running in circles but do form a system of lamellae, more or less parallel and arranged on the vessel wall one behind another. Most of them run obliquely or transversely to the long axis of the vessel. These lamellae grow outward from the vessel wall, and from the flow of the blood through a part of them, they become bent in the direction the blood is flowing. The appearance of this system is strikingly similar to the beautiful figures made on the fine sand of the seashore by the ebbing tide. The water pushes up the fine sand into ridges, until the strength of the flow overcomes the small obstacle, and the water travels on to build new ones further on. In this way then the beautiful series of ripple lines are drawn on the shore. The blood-stream builds up its lamellar system in an exactly similar way from the sand-like multitudes of blood-platelets. Zahn had previously taken notice of the work of de Candolle on the curious sand formations produced by moving water, and had put forward the suggestion that the lines visible on the surface of the thrombus are simply the effect of to and fro movements of waves of blood. But the essence of the matter is not that already existing sandy masses are shaped into systems by the movements of the blood, but that solid elements are taken from the flowing blood-stream and laid

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1. Derewenko: *Zieglers Beitr.*, 1910, *xlvi*, 123.

2. Ferge: *Med.-naturw. Arch.*, 1909, *ii*, 351.



down in the form of specially situated lamellae. I can only mention here in passing that by experiments which I carried out in the laboratory of Professor Rehbock I was able to find exactly similar deposits formed in running water from a given solid material, when the velocity of the flow was reduced sufficiently by the introduction of a dam or weir. No transformation of already laid down masses of particles was necessary to make the ridges, but the particles were laid down in such a way from the first. The question, of course, arises from these experiments as to why a deposit of platelets occurs at all, and this will be discussed later on. So long as the blood flows through this framework just as sea water flows beneath branches of a tree of coral, new masses of blood-platelets are laid down on the system already formed, and so new systems are always being built up behind the first one. The first formed system naturally grew faster, and finally there comes a time when the openings in the lamellae of the old part of the thrombus (this is indeed the *Kopfteil* which has already frequently been referred to) are so narrowed that the blood-stream becomes slower and slower, and finally stops altogether. With this event the building of a primary thrombus is finished, since once the blood-stream is at a standstill no new blood-platelets can be carried past, and hence further growth is impossible.

But before the complete cessation of the blood-stream occurs, a certain peculiar separation of the red and white corpuscles comes to pass, resulting in the whole system of platelets becoming covered with a layer of leukocytes. The facts of this peculiar separation are best explained by the well-known researches of Eberth and Schimmelbusch, who found that when the blood-stream was slowed the white corpuscles being of lighter specific gravity than the others, tended to travel at the margin of the blood-stream, and so are found closely in contact with the vessel wall. By the growing system of platelets which occurs in the formation of a thrombus the main blood-stream is divided up into a series of small streamlets. Each small streamlet is, however, subject to the same laws as a larger vessel, and when the slowing of the blood-stream occurs, the leukocytes are found close to the sides of each small streamlet, whose wall is in this case composed of the framework of blood-platelets.

Before I can turn to the question of why the blood platelets are deposited from the circulating blood, I must briefly describe the results which follow in the peripheral part of the vessel, when the stream is completely interrupted through the gradually increasing obstruction of a system of platelets. As soon as the lumen is closed in the region of the white, that is the primary thrombus, the whole blood-column becomes stationary right up to the place where the next anastomosing vessels enter, and undergoes very rapidly, as experiment shows, a complete coagulation. In this *Schwanzteil* of a thrombus, which is usually of some

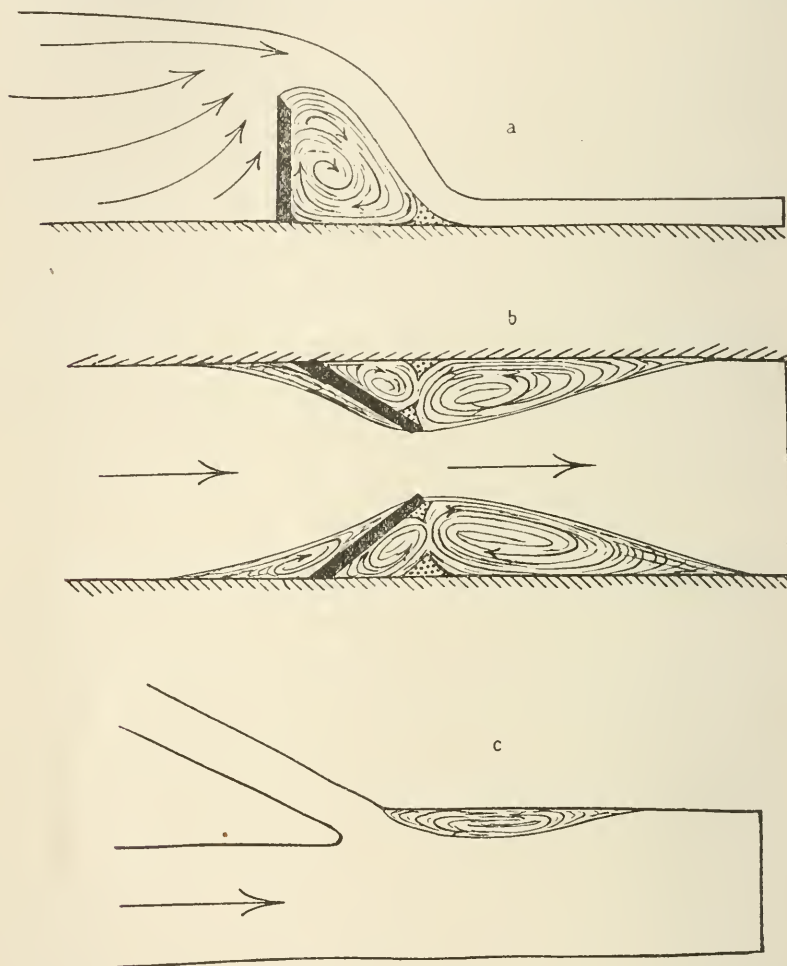
length and like the rest in color, we can expect, it goes without saying, no definite framework of platelets, and in fact it is either completely absent or occurs only partially. This red thrombus resembles in its microscopic structure essentially a post-mortem clot, and consists of an irregularly arranged mass of red and white corpuscles, blood-platelets and fibrin. It must be admitted that here the leukocytes and blood-platelets have a tendency to form themselves into masses, but there is no attempt at the formation of lamellae. The fibrin frequently shows striped thickenings running in definite directions. These may be the results of currents in the plasma, for although the column of blood has been checked it is still connected in manometer-like fashion with the rest of the vascular system. They may also merely be the expression of the movements of the fluids induced by the coagulation.

Thus an essential difference exists between the *Kopfteil* and *Schwanzteil* of the thrombus, which it must be said are connected together by a more or less transitional zone. This difference we know must depend on the fact that the *Kopfteil* is formed in flowing blood, the *Schwanzteil* in blood at a standstill. This brings up the question then of *why a white thrombus is built up from platelets in the flowing stream and a red thrombus when the blood-flow has ceased.*

#### MECHANICS OF THROMBUS FORMATION

After the corpuscular nature of white thrombi was recognized by Zahn, von Recklinghausen directed attention to the important bearing the slowing of, or rather the formation of eddies in the blood-stream, had on this question. His work has given considerable support to the well-known teaching of Virchow regarding the mechanical genesis of thrombi. When we compare the blood-stream with a river which has countless small particles of different specific gravity swimming about in its waters, we are driven to the conclusion that there must be some optimum velocity which will bring about a local aggregation of particles, such as we have seen by the microscope to occur in the building of thrombi. Neither by a rapidly flowing stream nor by complete stoppage of the current can such a heaping up be brought about. Thus the blood must flow slowly, or, to put it in a better way, must flow differently from the way it did before. It is noteworthy that deposits of sand in a river occur by preference where a widening or a deepening of the river bed exists. It is not a uniform slowing, but the inequality of the local conditions which predisposes to this extraordinary deposit. The insertion of a dam or barrier, by introducing counter currents and eddies, is also an important predisposing cause. I have made an effort to explain this building of sand banks on purely physical lines, and in this I have had the assistance of Prof. Dr. Rehbock in whose laboratory the experiments were

carried out. If a weir is introduced in a stream of fluid, then by the interference with the current so induced an unusually long "Walze" (or helix), as it is called technically, is produced in which a portion of the fluid flows backward. And not only that, but fine particles suspended in the water (we used sawdust for the purpose) all deposited in ripple



Diagrams showing (a) eddy formation behind a weir; (b) eddy formation in front of, behind and beneath obliquely-placed weirs; (c) eddy formed at the point of junction of two streams of unequal size. (From Aschoff: *Thrombose und Sandbankbildung*, Ziegler's Beitr., 1912, lii, 209.)

and net-like elevations in the regions of this "Walze." By means of obliquely placed weirs two "Walzen" can be produced, the one proximal, the other distal to the obstruction. It is at the point of contact of the two that thrombi arise, and, as Ferge also found, the thrombus is not built close under the valves of the veins. (See Fig. 1.)

Finally the question of sand-bank building by the flowing together of two unequal streams must be considered from the physical standpoint. When we study what occurs in a pipe when this state of affairs is brought about, it will be found that a slowing of the stream in the so-called zone of transition occurs. If the stream from the smaller vessel flows very slowly a sand-bank is formed at its mouth. This agrees very well with what is found in human pathology.

All these experiments are, on account of the number of factors outside our control, merely suggestive. We can take no account of the part played by the living blood and the living vessel wall. We can only prove the gross results and ask what alterations of the blood-stream, in the form of sudden slowing, backward flowing and eddy making, are found in the places where in the human subject autochthonous thrombi have a tendency to grow.

#### LOCAL CONDITIONS FAVORING THROMBOSIS

The special tendency to slowing of the blood-stream is quite properly insisted on by every one who has written on the subject. It is quite clear that some very special and unusual condition must be present in the arterial system to slow the blood long enough to allow a deposit of blood-platelets to occur. As Virchow pointed out long ago, and as is now generally recognized, there are certain situations in the venous systems which are especially predisposed to thrombus formation. Among these the veins of the leg, the proximal part of the femoral vein where the large valves are present, the pelvic plexus, the venous network of the dura mater and the auricles may be taken as examples. There are besides four conditions each of which, alone or in combination, has to do with the localization of thrombosis. First, there is continued overpressure on the wall of a vein, such as occurs in the veins of the leg from the pressure of the column of blood when the body is upright, or such as is brought about in the venous plexus of the pelvis by downward pressure of the intestines. This overpressure tends to physiological widening, and, finally, may terminate in a physiological thrombosis. I refer to thrombus formation in varicose veins in the lower limbs and to the thrombosis in the prostatic plexus in old age which is so usual as to be practically physiological. Thrombus building in the vaginal plexus, which may be the origin of a spreading thrombosis or be a nidus of secondary infection, has not had sufficient attention paid to it.

A second condition is the widening which obtains in the auricles, and also occurs in the veins at each valvular sinus. Ferger's recent experiments have confirmed the work of Kölliker and Epstein, who discovered that the musculature of the vein wall can be practically absent in the region of the valvular sinuses, so that backward pressure must



be followed by an ampulla-like widening above the valve. Further, the possibility of a backward pulsation in the veins, the so-called venous pulse, which often is most marked at the proximal end of the femoral vein, must not be lost sight of. Lastly, when the body is lying prone, certain local conditions affect directly certain veins with a known tendency to thrombosis. For example, the femoral vein just where the large valves are present, lies close under Poupart's ligament, and the importance of the bend so brought about must not be underestimated. The iliac vein shortly after the junction of the hypogastric vein, also comes into the same category, and its tendency to thrombosis is also to be referred to the bend in the path in which the blood flows.

Naturally, these local factors play an even greater part when any defect in the heart's action leads to a general slowing in the venous system. In such a case the posture of the patient exerts a further and additional influence. In certain conditions the posture has directly to do with the left or right sided position of the thrombus, the limb which lies lower having a greater tendency to become thrombosed. When lying on the back the increased compression of the left iliac vein by the arterial trunks (right iliac, middle sacral and left hypogastric arteries) has a direct influence in slowing the stream, and explains the well-known frequency of thrombosis in the left lower limb. Another point, too, is of interest. When a double sided thrombosis of the veins of the lower limbs occurs, the following characteristic conditions can often be found. The thrombosis on the right side extends up to Poupart's ligament, whereas on the left side it extends up to the point of compression of the left iliac vein by the right iliac artery.

The deposition of blood-platelets in all the above mentioned examples of thrombosis is easily understood. It is not the stagnation but the retardation which brings about the thrombosis, and must be reckoned as the direct cause of the deposition. Eberth and Schimmelbusch have given to this type the name of conglutination thrombosis. They took this descriptive name from a phenomenon which occurs in the heaped-up mass of platelets, namely, the cementing of the platelets together. This phenomenon can also quite truly be called agglutination, so the term agglutination thrombosis is also a suitable one.

Between accumulation of platelets on the one hand and conglutination or agglutination on the other there exist very special relationships.

We do not know as yet anything of the phenomena which precede agglutination and which are the very earliest factors in thrombosis. We cannot say whether they are of a type long known to workers in bacteriology or serology, or whether they are chemicophysical. We suppose at least that a special degree of viscosity must be present to allow the masses of platelets to form so easily, whereas precipitation phenomena

in the sense of agglutination can also play a rôle. It is also quite conceivable that a mass of platelets, once it is formed, can exert an influence on other platelets flowing past it. The agglutinability of the platelets depends, of course, on the chemicophysical characters of the fluid in which they are, in this case, the blood-plasma.

Thus we see that alteration in the blood-stream is only one condition in the making of a thrombosis, the second condition being dependent on the agglutinability of the platelets themselves. Unfortunately, we have no knowledge of spontaneous changes occurring in this property of agglutinability. This question, chiefly from the experimental side, is considered by Achard and Aynaud.

It is evident that the number of the platelets is an important matter to be considered in relation to thrombosis. Judging from the literature it seems clear that the number of platelets—their exact enumeration is no light matter—undergoes great variations in cachectic states. We know too, that after experimental anemia the number of blood-platelets increases rapidly, coincident, as has been shown by Ogata, with increase in the number of giant cells in the marrow. It is, therefore, quite feasible to suppose that subsequent to loss of blood at operations or during parturition, an increased tendency to thrombosis can be brought about, although it cannot at all be said that an increase in the number of platelets can by itself alone bring on thrombosis.

#### VASCULAR CHANGES AS INFLUENCING THROMBOSIS

We have up to this point considered two of the conditions which have to do with the building of thrombi, namely, changes in the blood-stream, and qualitative and quantitative alterations in the platelets. We will now take up a third condition, which used to play the chief rôle in the teaching about thrombosis, but whose signification was soon greatly limited by Virchow. I refer to alteration of the vessel wall itself. That this is not the decisive, not to say the only cause of thrombosis of the ordinary type, is made very clear when we can think of an atherosclerotic aorta, which in spite of the most marked changes can remain quite free from thrombi. Here, of course, there is no slowing of the blood-stream. On the other hand, the question arises whether the slowing of the stream can alone, without any accompanying change in the vessel wall, give rise to a thrombosis by deposition. Do the deposited platelets remain bound together only when the endothelium is damaged? Is this damage of the endothelium a third condition which must be fulfilled before thrombosis by deposition can arise, and how must the endothelium be damaged? Covering this point of endothelial damage, the importance of which is always accepted without question, and which is always given great prominence in the literature, we know in reality practically nothing. We

can affirm only one thing, namely, that chronic changes of slight degree in the intimal lining of a vein, as for example fatty changes of the endothelium, can by themselves play no important rôle, since one must so often assume this change to be present without thrombosis resulting. Besides, it is very difficult to understand how a fatty change of the endothelium merely can lead to a deposition of blood-platelets. I can find in the literature no well authenticated cases, in which thrombosis has really been brought about by changes in the endothelium, a factor which is regarded by many authors as of special importance. It is naturally quite another story when rigidity of the wall and valves of the vein, brought about by severe and progressive phlebosclerosis, has interfered with the blood-flow. Changes in the wall of the vein are then not the direct, but only an indirect source of thrombus formation. An analogous state is brought about in the numerous experiments carried out to induce thrombosis by means of corrosives, cutting and the introduction of threads or other foreign elements. These experiments were carried out at an early date by Zahn, Eberth and Schimmelbusch, Welch, and more recently especially by Zurhellen in the institute in Freiburg. Here the rough and mechanical disturbances of the blood-stream resulting from the laceration of the vessel wall, do not play the entire rôle, but the plasmatic exchanges between the fluids of the blood and the lining of the vessel must also be important. When instead of slow retrogressive changes, the intima is suddenly stripped of its endothelial lining, then a reaction inevitably ensues, as occurs in all living tissues, accompanied by a pathological flow of lymph. Then it is, indeed, the alterations in the blood-stream in the region of the injured area which bring about a thrombosis by deposition. It is not made clear by the previous experiments how and whether a direct influence on the blood-platelets as they flow by can possibly be exerted by agencies of an agglutinative or coagulative nature produced by the tissues ( L. Loeb, Achard, Aynaud). Nor is it explained whether the connective tissues or the muscle laid bare by the damage at a surface capable of absorption (Morawitz) can bring about the adhesion of the platelets. At any rate, all these artificially produced thrombi present the characteristic structure of thrombi by deposition of the blood-platelets. We must allow that up to the present we have not been well enough informed concerning the first and absolute beginnings of thrombus formation in the case of autochthonous thrombi in the human subject. Unfortunately in this connection we can hardly ever obtain fresh enough material, since, of course, we must eliminate all post-mortem coagulation, which naturally can occur in the thrombus itself.

We must endeavor to get a clear idea of why the adhesion of the platelets to the vessel wall occurs in autochthonous thrombosis, since we

assume that this deposition of platelets is the first procedure. It may be quite possible that the endothelium dies as a consequence of being covered over by the platelets. Fibrin is in this way set free and cements the masses of platelets to the wall. Or the platelets after lying on the wall for some time may themselves die, and then coagulation on and between the endothelial cells can ensue. At any rate, it is necessary that an interaction of a certain duration occur between the platelets and the endothelium in order that the material may remain stationary and, so to say, be fixed there. If slowing of the blood-stream and alteration in the conditions of the platelets are to figure as the direct facts in thrombus formation, then we must consider as indirect factors changes in the wall, alterations in the cardiac action and loss of blood during operations.

In considering the vital question, as to why a white thrombus occurs in the circulating blood, and a red thrombus only when the stream is stationary, I have already attempted to answer the first part and shall now proceed to the second. I have already mentioned that a red thrombus arises only when the lumen of the vessel is sufficiently closed by a white thrombus to bring the blood-stream to a standstill in the peripheral portion of the vein. This stationary column of blood coagulates. But under the microscope this red thrombus, dense in the neighborhood of the *Kopfteil*, while peripherally becoming more and more spongy, and, finally, almost fluid in consistence, can scarcely be differentiated from postmortem clotting. The microscope shows, however, that the denser portion is richer in fibrin and leukocytes, and suggestions of lamellae can here and there be recognized. As we travel more to the periphery the structure of the thrombotic mass more and more approaches that of the normal blood. Microscopically, it is also true that the boundary between the white and the red portions is as hard to recognize as that between the slowing and final complete stoppage of the blood-stream. From these points we can also explain why this column of stagnating blood does go on to coagulation, in contradiction to the well-known findings of Baumgarten, who observed that the column of blood contained in a doubly ligated portion of a vessel did not coagulate. We must take into consideration that, as has been already mentioned, coagulation phenomena can also be observed to have taken place in the blood found occupying the gaps in the white portion of a thrombus. These phenomena very probably must be referred back, judging from the characteristic situation of the fibrin network, to an interaction between the fibrin ferment set free by the death of the platelets and the stationary blood-plasma round about them.

Of course, the amount of fibrin ferment set free in the white *Kopfteil* will be very considerable. It can advance by diffusion into the caudal red portion of the stagnating blood-column, which is richer in leukocytes



and platelets and develop its action. We know, as Baumgarten has stated, that the cause of the blood remaining fluid in a doubly ligated segment is that the blood dies relatively slowly. Conversely, we can presuppose a very rapid death to occur in a structure rich in ferment, as the blood-platelets are, and so the clotting of a column of blood in stagnation becomes intelligible. I will not consider here the processes which precede clotting, and above all I cannot discuss the question of where the forces which bring about clotting have their origin. Nor can I stop to inquire whether the source of thrombokinas is to be sought for only in the thrombocytes and leukocytes, or in the erythrocytes as well. I would prefer to refer you to the well known and very clearly expressed papers of Morawitz.<sup>3</sup>

I pass over here the finer morphology of the processes preceding clotting, and would only mention that the "centers of coagulation," so-called, are to be regarded in my opinion as acting both chemically and physically. In the origin of the red thrombus we are also dealing not with the separation of the formed elements of the blood, which practically does not occur, but more especially with crystallization of fibrin as in ordinary clotting. Thus we have a coagulation of the blood as a whole, and not merely the deposition of some parts of it. This view has already been suggested by previous authors, who have put forward the name coagulation thrombosis, in contra-distinction to conglutination and agglutination thrombosis.

Just as in deposition thrombosis the slowing of the blood-stream and the relations of the platelets as to number and viscosity are the essentials, so in clotting "*en masse*" the stoppage of the stream and the increase of fibrin ferment are the important factors. It cannot be denied that an intimate relationship exists between the heaping up of platelets, the agglutination and the clotting, i. e., the coagulation. He must conclude that agglutination is, so to speak, only a means of creating in the circulating blood conditions which are necessary to allow of the crystallizing out of fibrin, a process which cannot occur in the circulating blood under ordinary conditions.

But it is quite evident that these two phenomena (agglutination and crystallizing out of fibrin) do not mean the same thing and must be kept genetically apart, since each can occur without the other. In the case of ordinary autochthonous thrombosis, with which we have been dealing up till now, the deposition and coagulation processes are closely related. The process of deposition brings about the coagulation, and is, so to speak, the indirect source of it. Then the further question arises as to whether coagulation thrombosis can occur apart from the process of deposition, and what may be the indirect causes influencing them both.

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3. Morawitz: Blutgerinnung, Handb. d. Biochemie d. Menschen u. d. Tiere, 1908.

It is important in this connection to remember that while thrombosis by deposition can be brought about readily at any time, a primary coagulation thrombosis is very difficult to induce. The stoppage of the blood-stream alone does not suffice, as Baumgarten's experiments have shown, for the ferment does not develop quickly enough. Also optimal relations must be established between the rate of stream and amount of ferment present, to initiate the process of clotting. Even the flowing blood, however, can be made to clot, if a large quantity of ferment is introduced all at once, either by the injection of pure fibrin ferment or by artificial transfusion. Of course, control by the microscope is essential in such experiments to ascertain whether the solidification of the blood depends really on clotting of fibrin, and is not merely a precipitation, such as occurs when a substance which coagulates albumin is introduced.

To the question of these precipitation thromboses, and other related processes, I will return later.

Two other processes must be mentioned here which undoubtedly have relationships with coagulation thrombosis. I refer to ligature and infection. Since, as we shall see, both of these can also induce thrombosis by deposition, we can now inquire how ligature and infection, respectively, act, and what is their significance from the point of view of thrombosis.

The facts in the case of ligature are comparatively simple. If done carefully so that considerable damage to the intima is avoided, and the portion included by the ligature is so small as not to interfere with the movement of the blood, no clotting need necessarily occur, a fact which we have noticed elsewhere in connection with transplantation of blood-vessels. At other times we find thrombus formation, often quite scantily, however, in the region of the intimal damage, where the fibrin ferment arising from the injured wall can act on the stationary blood in its neighborhood. Or at the margin of the ligatured part of the vessel a true deposition thrombosis may arise as a result of local eddy formation. In this way the area may finally be separated off completely from the blood-stream, and the column of blood thus shut off passes into a condition of coagulation thrombosis, like the *Schwanzteil* of an autochthonous thrombus.

#### RELATION OF INFECTION TO THROMBOSIS

Much more difficult to explain is the question of infection to thrombosis, nowadays considered so important. It is especially from the findings of clinicians that the idea has been put forward that not only most, but as some will have it, all forms of thrombus formation are to be referred back to infection. It is unfortunate for the advancement of pathological anatomy that when as a sequel of thrombosis cases come to autopsy, an infection often does not exist or at any rate can no longer be

recognized. Concerning infective thrombosis, I would say only that from my own researches I am of opinion that what occurs generally is a secondary infection of an already existing simple thrombus; for example, in the site of an operation or in the area of uterine wall laid bare by the removal of the placenta. Of course, it is not impossible that a primary inflammatory process in the surrounding tissues can implicate and extend through the wall of a vein and a thrombosis occur from the changes in the blood-stream arising in that way. Of more importance, however, than these locally infective thrombi are those of septic character which arise at a distance from the site of an operation; for example, septic thrombosis of the femoral vein following operative interference with the pelvic connective tissue. Here our observations have shown that a typical thrombosis by deposition always occurs, the organisms themselves being found enclosed here and there in the framework of the thrombus. One must necessarily conclude from this histological picture that the organisms circulating in the blood are only, so to speak, secondarily enclosed in the thrombus while it is being built. The thrombus is otherwise identical in structure with a simple one. We must, therefore, assume that here the thrombosis has a similar origin as in the non-infective type, namely, a slowing of the blood-stream, and that the organisms themselves are not the causative agents. The latter are merely accidentally enclosed, but are dangerous, since by their multiplication they convert a simple thrombus into a septic one, and may bring on all the consequences of septic emboli. The following abridged account may be given of the complicated picture which is produced by the infection occurring in the thrombus in a vein.

1. A local infective thrombosis in the region of an operation or in the neighborhood of the placenta can arise: (a) Through infection of thrombi already present, to which an inflammation of the wall has arisen secondarily (thrombophlebitis). (b) By an infection progressing along the vessel wall, which induces first of all a primary phlebitis and is followed by a secondary thrombosis by deposition (phlebitic thrombosis). (c) By means of slowing of the blood-stream and thrombus formation in the commencements of the veins followed by thrombosis by deposition in the larger veins, induced by toxic or inflammatory causes, which subsequently become infected.

2. An infective embolic thrombosis arising at a distance is in most cases the sequel of an ordinary primary thrombosis, which acquires its infective character secondarily by multiplication of micro-organisms in the blood shut in the interior of the thrombus. Experimentally it would appear probable that only exceptionally the organisms separate themselves from the blood, directly infect the wall and so bring about a secondary thrombosis.

3. All septic thrombi can extend on account of the inflammatory changes in the wall. These are not confined to the area in contact with the clot, owing to the toxins being diffusible.

Since in the case of distant thrombosis the infection usually arises in already existing thrombi, and cannot be regarded as the source of the thrombosis, but rather as an accompanying or induced phenomenon, we

must inquire why, especially after operative procedures or in already established infection, thrombosis at a distance is of frequent occurrence. It is well known that in acutely progressing septic cases thrombosis at a distance is much rarer than in subacute or chronic cases. That in itself speaks against a direct action of infection.

Thrombosis of the femoral vein, among the thromboses at a distance, interests us most, since it is the special origin of fatal lung emboli. Here extremely varying factors work together—loss of blood at operation, weakening of the heart, general prostration, confinement to bed, the influencing which every pelvic operation has on the return of the venous blood from the lower limbs to the heart, because the venous plexus of the pelvis acts as a manometer to this great area of blood.

Finally, the possible changes in blood platelets themselves induced by the infection must also be considered. In reality we have no positive knowledge on the question whether the bacterial toxins can increase the viscosity of the blood-platelets.

I have taken up so much time with the static or autochthonous thrombosis, because it seems to be the most important variety, and is, as a rule, easily recognized both by the clinician and the pathologist. With its description, however, the question of thrombosis is in no way exhausted. I have already made special mention of the work of my American colleagues, Flexner and Loeb, who, by using toxins, studied the processes in the living body which accompany the toxic thrombosis described previously by Naunyn, Landois, Ponfick, Silbermann, Kaufmann, Falkenberg and others, as resulting from blood transfusion and specific blood-poisons, and by Takowski, Lubarsch, Bardeleben, etc., as a consequence of injection of bacteria and bacterial toxins. Recently in Germany, Dietrich and my own pupil, Kusama, have carried out such experiments, and I would like to make a few remarks about Kusama's work which has only lately been completed.

#### CAPILLARY THROMBOSIS

Since the independent elements of the blood are influenced in the highest degree in all these thromboses of toxic or infective origin, while the conditions in the blood-stream itself remain essentially the same, I have called this form of thrombosis "thrombosis by alteration of the blood," in contradistinction to "static thrombosis." Further, as you will hear immediately, the initial thrombus building occurs in the capillary system, and so one can also call this form capillary thrombosis, in contradistinction to that occurring in the larger vessels.

In continuation of the work of Flexner and Loeb, who, by injecting heterologous serums, have explained the significance of the agglutination of erythrocytes, the deposition of platelets and the formation of fibrin



in thrombosis, and in connection also with the work of Dietrich, who, above all, has tried to elucidate the mode of action of the end-products of hemolysis, namely, the blood-cells on one side, and the agglutinins and precipitins on the other, my pupils and I have tried to recognize the factors which are essential for the production of thrombi in the circulating blood.

So far, our experiments agree more with those of Flexner and Loeb than with those of Dietrich, who studied chiefly the effects on the stationary blood-column. We began by investigating the effects of homologous serums on rabbits. I shall take no notice here of the clinical symptoms induced nor of the altered coagulability of the blood, but would chiefly confine myself to the morphological findings. Rabbits showing no signs of illness, were killed twenty minutes after the injection of a homologous serum. Examinations of the organs just after death showed in the places predisposed to capillary thrombosis—for example, the capillaries and post-capillary venules of the lungs—very abundant thrombus formation. The thrombi consisted in parts exclusively of blood-platelets, packed together but well preserved, in other parts of a mixture of platelets and red-cell stromata. When the fixation was good, fibrin was absolutely absent or only to be found in traces inside the thrombi. On what, then, do these thrombi, formed from platelets and red-cell stromata, depend? They might be a direct effect of the serum injected, which both agglutinated the platelets, and after hemolyzing the erythrocytes, agglutinated their stromata. To answer the question, Kusama hemolyzed red corpuscles by washing them thoroughly with distilled water and then injected the solution of hemoglobin. When the animals were killed, ten to twenty minutes after such an injection, thrombi composed of blood-platelets could be found in the vessels of the lungs. On the other hand no thrombi composed of stromata were met with. A strongly agglutinative effect on the blood-platelets is thus exercised by hemoglobin circulating free in the plasma. It is noteworthy that in these experiments the platelets also remain very well preserved. By simple washing of the stromata with distilled water a distinct effect on them is produced. If a suspension of these were injected into rabbits and the animals killed after a definite time, thrombi composed of stromata were not only found to have arisen by the agglutination of these cell remnants, but also undoubted thrombi from blood-platelets were found. The increased agglutinability of the platelets must have been brought about by living substances produced by the solution of stromata in the blood plasma. If a suspension of stromata, which had been heated at 56 to 58 C. for forty minutes were injected, no such blood-platelet thrombosis ensues. So it seems that the living substances set free by the dissolving of the stromata in the plasma, are of great

importance in effecting an increased agglutinability of the platelets. Experiments in animals which were allowed to live a longer period show that the stromata do dissolve in the circulating blood. In such experiments thrombi composed of stromata were found to have completely disappeared, so they must either have dissolved, or as is much less likely, have been washed away by the blood-stream. Thrombi composed of blood-platelets are also not seen in these animals, and from the fact that the individual plaques were still well preserved, it seems reasonable to suppose that they again enter the circulation.

Now let us consider how a foreign or heterologous serum acts. In Loeb's work, dog's serum was taken as the representative of strongly hemolytic and coagulating serums, while ox serum represented the group of strongly agglutinative serums. After the injection of dog's serum the rabbits died within a few minutes, showing characteristic symptoms (excitements, coma, convulsions, projecting eyeballs, abdominal distention, etc.). At the autopsy, fresh clotting was found in the lung arteries, in the right side of the heart, in the vena cava and in the lung veins, and death appeared certainly the result of these pathological findings. When, however, the experiment was carried out, after carefully opening the pericardium, it was seen that the clinical symptoms and the stoppage of breathing occurred while the heart continued to beat and the blood remained quite fluid. Microscopically, just as in the case of injection of a homologous serum, thrombi composed of stromata and platelets are to be seen in the lung capillaries, in the post-capillary venules, and also in the precapillary arterioles. But these thrombi differ from those discussed before in that the platelets show definite disintegration. Thus, not only agglutination, but destructive processes have been produced. This latter occurrence can only be caused by the heterologous serum itself, and the marked increase in the coagulability of the blood must also be referred to the disintegration of the platelets. One might be inclined to think that death might be produced by the blood-platelet thrombi, whereas the coagulation of the blood alone could not be the cause. But in this connection it is easy to show that where an animal has been given a non-lethal dose of dog's serum and been killed many hours later, capillary thromboses are just as marked as when the animal is killed at a much earlier stage. So, then, the fatal result can be referred neither to the blood coagulation nor to the capillary thrombosis. It must depend on some other changes in the blood, or increased viscosity of the plasma, or direct toxic action on the central nervous system.

The fact proved by Loeb's experiments with leech extract (hirudin) that the clotting is not responsible for death, was also confirmed by Kusama. Many rabbits, after injection of hirudin, died, although the blood postmortem was found quite fluid. The blood platelets seem to be

diminished, since, according to all accounts, hirudin has a hindering action on the hemolytic and agglutinative properties of a heterologous serum. Experiments with ox serum give quite similar results. Kusama was unable to confirm the difference on which Loeb laid emphasis, namely, that by injection of ox serum marked clotting is absent. But he fully confirmed another point on which Loeb laid stress, the fact that agglutination plays a special rôle in all these experiments. In contrast to what occurs with dog's serum, many thrombi formed from stromata are found, but few composed of blood platelets. Also the platelets are not altered in the same way as after injection of dog's serum. It should be explained in addition that in some of the experiments with ox serum the coagulability of the blood did not show nearly so marked an increase as after injection of dog's serum.

Since as a matter of fact blood-platelet thrombosis, which in most researches has been given only a subsidiary place, is a result of hemolysis, we should be able to reproduce a similar thrombosis by injecting other hemolytic agents.

With this end in view, researches with ether were undertaken. It was previously believed that injection of ether was followed by a direct clotting of the blood, but as the result of experiments carried out by myself I have been opposed to this view for a long time. Then Loeb showed that injection of ether brought about an agglutination and destruction of erythrocytes, and considered the agglutination the cause of the death of the red cells. Our own experiments confirm this in so far that ether is hemolytic just like dog's serum, and brings about thrombosis by deposition of stromata and platelets. But no peculiar or special red-cell thrombosis is called out by its injection. Ether is in addition directly destructive to the blood-platelets, and so the increased coagulability of the blood is explained. Glycerin and distilled water act in a manner similar to ether.

On the contrary, other blood-poisons, ricin for example, act in a surprising and entirely different way. Kusama's experiments showed that practically no hemolysis of erythrocytes occurred. Hence no noticeable thrombosis from blood-platelets or stromata ensued.

What does occur is really this: Ricin acts destructively on the myeloid and lymphoid cells of the spleen, bone-marrow and liver, and on the endothelial lining of the vessels. The fragments of cells so produced pack together in the capillaries of the spleen, bone-marrow and liver, and induce thrombosis. A definite increase in the coagulability of the blood does not occur as a rule. The coagulation of fibrin is secondary, just as in the case of thrombosis from stromata and blood-platelets.

Finally, experiments with bacterial cultures seem to be especially important. By injecting dead cultures of the *Bacillus typhosus* a definite

fibrin thrombosis was produced, especially in the lung capillaries, but also in the capillaries of many other organs. In these thrombi leukocytes were enclosed, either broken down or well preserved. Destruction of myeloid and lymphatic cells was seen in the hemopoietic system, but not nearly to such a degree as after ricin. It was very strikingly brought out that these small thrombi from cell fragments (Trümmerthrombi) were accompanied by such a considerable deposit of fibrin. It seemed as if to explain it the bacteria must also be considered as foreign bodies. It is very difficult to extract the endotoxin of the *Bacillus typhosus*, so the experiment could only be repeated with typhoid bacilli heated for a much longer time. The results were similar, but not nearly so marked as when bacilli only killed by heat were injected. Also a previous immunizing of the animal against *Bacillus typhosus* had no obvious effect in decreasing the amount of thrombus building and of fibrin deposition. Dead cultures of the bacillus of dysentery and of staphylococci behave in exactly analogous fashion. In the case of cultures of the latter organism the destructive action on the cells of hemopoietic organs is very slight, whereas the formation of thrombi from leukocytes and fibrin in the capillaries of the lungs and other organs is very marked. The leukocytes always contained very numerous cocci.

In conclusion, I would say that the effect of bacterial injection is merely the production of leukocytic thrombi, and deposition of fibrin follows closely on this process. The definite occurrence of leukocytic thrombi, although they are only temporary, explains the marked leukopenia which can be recognized one to four hours after the injection, in the peripheral blood, in most of the experiments.

To decide what part of the process could be referred to the mere presence of foreign elements in the blood, other animals were injected with a solution of India ink. The result was a definite thrombosis from blood-platelets in the lung capillaries. Many leukocytes were enclosed in the thrombus, but no *deposit of fibrin* occurred. This result is different from that produced by bacteria, in that deposition of fibrin was absent, a blood-platelet thrombosis being the chief result. No marked destruction of leukocytes was seen. Phagocytosis of particles of India ink, although it was present, was not so marked as in the case of cocci. The packing of the leukocytes inside the lung capillaries produced a leukopenia just as after injection of dead bacterial cultures.

Collargol was also injected and produced a thrombosis chiefly from blood-platelets, agglutination of leukocytes being practically absent. Olive oil gave a similar picture. Injection of ground rice was followed by the same result induced by bacteria, in that, whereas blood-platelet thrombosis did not occur, thrombi from leukocytes and fibrin were to be found in the lung capillaries.



Thus all foreign elements do not act in the same way. Substances like India ink, collargol and olive oil must have some attraction for platelets, whereas the ground rice and dead bacteria have none. The first three produce thrombosis from blood-platelets, the latter groups only leukocytic thrombosis and the greater the destruction of leukocytes and platelets the sooner is fibrin deposited.

#### CONCLUSIONS

What result, then, can we glean from all these experiments? We have seen that different poisons, whether hemolytic in action or not, bring about distinctive forms of capillary thrombosis, among which we can differentiate the following types:

*I. Blood-Platelet Thrombosis.*—This arises by intravenous injection of a homologous serum, of solutions of hemoglobin and stromata from homologous blood, of heterologous serums, ether, glycerin, distilled water, India ink, collargol and olive oil. The thrombi arise in the capillaries of lungs, liver and spleen.

*II. Thrombosis from Blood-Stromata.*—This occurs in pure form only, when very marked hemolysis occurs in the blood-stream as a result of the action of hemolytic agents. Otherwise it occurs only in conjunction with blood-platelet thrombosis.

*III. Thrombosis from Fragments of the Blood Elements (Bluttrümmerthrombose).*—Disintegration of the elements of the blood occurs by introduction of ricin, bacterial toxins, etc., which destroy the leukocytes, lymphocytes, macrophages of the spleen, liver and bone-marrow.

The fragments, in conjunction with scanty fragments of erythrocytes, roll themselves together to form thrombi in the spleen and liver. Fibrin is then subsequently deposited there.

*IV. Leukocytic Thrombosis.*—The polymorphic and mononuclear leukocytes phagocyte the foreign elements introduced into the blood-stream, and are stopped and held back in the capillaries of the internal organs, especially the lungs, spleen and liver, so that a leukopenia results in the peripheral circulation. This process is especially brought out by bacteria, but also by various indifferent foreign substances like particles of ink and starch.

*V. Fibrin Thrombosis.*—Although by injecting stromata or foreign serums the coagulability of the blood can be increased, no deposit of fibrin occurs. Deposit of fibrin occurs only secondarily, as a sequel of the types of thrombosis already described.

Let us take, then, a final glance over the subject of static and toxic thrombosis. We see that neither endothelial damage, on which so much stress was previously laid, nor a direct coagulation of the blood, play any rôle. Only two real reasons for the thrombosis require to be con-

sidered, and their merits weighed one against the other. I refer to the changes in the blood elements themselves, and to the slowing of the blood-stream. Neither of these two factors can be excluded, as even in the experiments where thrombi of different sorts are produced by heightening the agglutinability of the blood-platelets by the disintegration of white or red corpuscles, these thrombi can occur only where *normally* a marked slowing of the blood-stream is present; for example, in the capillaries of the lungs, liver and spleen. But even by very marked destruction of the blood elements it is impossible to produce thrombi by deposition in the larger veins when no slowing of the current occurs.

An additional factor, therefore, needs to be considered, especially in cases in which the thrombi are localized in the large veins, and where the above-mentioned capillary regions of the lungs, liver and spleen generally implicated in toxic thrombosis remain free. In such a case the increased agglutinability of the blood-platelets cannot be the direct exciting cause, but a definite and recognizable slowing of the blood-stream must first of all occur.

So we see from all these experiments that the slowing of the blood-stream and the alteration of the blood elements themselves, especially alterations of the platelets, are the chief factors in the production, not only of the static, but of toxic thrombosis, so far as we are concerned with thrombus formation in the flowing blood-stream. In static and similar types of thrombi the slowing of the blood-stream is of prime importance, while for the toxic varieties the changes in the blood elements have the dominating influence.

Nevertheless, we must ask ourselves whether very important and intimate relationship do not exist between thrombosis and coagulation of fibrin. There is no doubt that, with a few exceptions, coagulation of fibrin as a rule sooner or later inevitably follows thromboses. So the division of the process of thrombosis, as set forth by Loeb for the lower animals, is now quite clear. The first stage is the erection of a morphological structure by a process of *agglutination*. Fibrin ferment is obtained from the agglutinated elements and cements them together by coagulation. This is the second phase.

Therefore, in the process of thrombosis in human beings the phylogenetic development of the complicated mechanism of coagulation is repeated.

## STUDIES OF BLOOD-PRESSURE IN STATES OF EXCITEMENT AND DEPRESSION \*

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This paper treats of blood-pressure, work done recently under the direction of Dr. H. D. Singer, at the State Psychopathic Institute, in cases of mania, depression and agitation. The sphygmomanometer used was that of Erlanger. The number of cases investigated is not large, neither do the extremely-marked examples of mania or agitation figure among those cited because it is impossible to get a reading in such cases. The reason is that as one is dealing with a delicate and sensitive machine which registers blood-pressure graphically, the procedure would probably result in disaster and the reading be worthless. When the typical is sifted out and the impossible is eliminated, surprisingly few cases are left to be dealt with. These few cases, then, were taken without regard to the age of the patient or to the condition of the vascular system; the readings were all taken at regular intervals and at the same time of day, and the patients were all in the same position, namely, lying on the operating-table. In fact, as far as possible, all the conditions were made constant in each one of the cases. Several readings from the same patient were taken, and this I deem quite important, as we find that the blood-pressure at different times and in some patients varies quite considerably. No accurate deductions could be made from one reading. One might quite erroneously consider a systolic pressure of 200 mm., such as was seen in one of the cases of depression, as a complete confirmation of the accepted view that in cases of depression high readings are obtained and, therefore, take no other reading, when as a matter of fact a day or so later the systolic pressure in this same patient was 140 mm. Again, having the graphic representation of the blood-pressure, one can quite accurately ascertain the diastolic as well as the systolic pressure, and thus find the pulse-pressure quite an important thing to have recorded. The graphic method also records the amplitude of the wave. The observation of this has led to certain views concerning the tracings which will be taken up later. Some marked variations are noticeable.

Having obliterated the pulse by the inflation of the cuff, the air was allowed slowly to escape until the needle began to oscillate when the

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\* From the Illinois State Psychopathic Institute; read before the Illinois State Hospitals Medical Association, Elgin, Ill., April 24, 1913.

\* Submitted for publication June 30, 1913.

height of the mercury in the tube was taken. The finger also was used to catch the returning pulse, and in each instance the pulse was caught after the other observation was made. The difference in time between these two observations decreases as the blood-pressure rises. Hence, it might be said that the readings quoted are slightly higher than they would have been had we used other types of sphygmomanometer. The time is given in seconds measured by an electric metronome.

In reviewing the literature I find in Ziehen's "Lehrbuch" several references which will be considered. Ziehen accepts what Dr. Craig<sup>1</sup> has to say. The latter investigator conducted several experiments to find out first, whether or not certain forms of insanity fall under different heads as far as blood-pressure is concerned, and second, if they can be arranged in different groups, whether the altered blood-pressure is a cause or a result. He discovered that in a general way insanities fall into two groups, namely, that persons under excitement have a low pressure and those under depression have a high pressure. His investigations were all made at the same time of day; no drugs were used; the condition of the bowels was noted, and the cases of known vascular and kidney disturbance were eliminated. For check, he used normal persons with blood-pressures of 120 to 125 mm., and even here he found variations. For instance, those of excitable temperament gave lower and those of apathetic temperament higher pressures. In twenty-one women with acute melancholia he found a pressure of 150 mm. In fifteen men with acute melancholia he found a pressure of 140 to 145 mm. In twenty-two excited women patients he found a pressure of 105 to 110 mm. In eleven excited men patients, pressures of 105 to 110 mm., and some as low as 95 and 100 mm., were found. He cites one case of depression with a blood-pressure of 150, which, on recovery, dropped to 125; this was followed by an excited period with a pressure of 105; recovery ensued and the pressure rose to 125 mm.

In agitation with extreme motor restlessness the pressure is, as a rule, low. In stupor it is high, but in the depression which follows acute mania, which he interprets as an exhaustive condition, he found on the contrary, low pressure. In paretics the pressure is raised during depression and lowered during excitement. In fact, it seems that in all cases of affective disturbance there are oscillations of pressure, whereas in cases of paranoia, where delusions are only ideational, there are no variations from normal. He recites cases of aortic regurgitation (low pressure) in which the patients, on becoming insane, usually presented manic states, while conversely in cases of mitral regurgitation (high pressure) the insanity takes the form of melancholy.

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1. Craig: *Lancet*, London, June 25, 1898.



While no definite conclusions are made as to a solution of the problem, he considers blood-pressure *per se* is not the cause of insanity; but suggests that insanity and blood-pressure may have a cause in common and cites a case in which the pressure fell *pari passu* as the excitement rose. Conversely, there is no definite proof that insanity causes a change in blood-pressure, and, as an example, he cites paranoia. He considers autointoxication in its causative relations and cites the feelings of depression induced by constipation, and the relief not only of depression but also of blood-pressure, particularly in melancholia, when the constipation is relieved. This is on the theory that the splanchnic system regulates blood-pressure in the brain.

Pilez<sup>2</sup> quotes Gärtner, whose sphygmomanometer he used, as saying that emotional excitement raises blood-pressure and that the first reading is usually the highest because of the stimulation which results from the procedure — a statement which he can confirm — and adds that certain definite mental diseases were marked exceptions to the rule. In each case he took two readings to allow for psychic influence, except in paretics, who were so demented that they were psychically uninfluenced. Normal pressure Pilez estimates at 105 to 130 mm. Two hundred and forty cases were taken under observation, and nine hundred to nine hundred and fifty readings were made. Assistants, attendants and paranoiacs were used as controls. Paretics gave normal readings at first, but as dementia progressed the pressure fell as low as 50 in bed-ridden patients. Patients in the depression of paresis gave higher readings. By the pressure-fall in the terminal stages of paresis one is able to prognosticate death, even though at the beginning decided arteriosclerosis were present with very high pressure. In depressed patients he found high pressure, but in simple cases the readings were still within normal limits, though relatively high. This was also true of cases of agitation when under the influence of morphin and in the interval between paroxysms. In circular insanity, patients when depressed gave high readings, and when excited low readings. When a reverse reading was obtained it would seem to prognosticate an on-coming variation in mood. He calls attention to Craig's statement, which he does not understand, that in extreme cases of anxiety the blood-pressure goes down, but agrees with him that the exhaustive stupor following mania gives a low pressure. In stupor, following melancholia, the author found high pressure, but no change in catatonic stupor.

Kramer<sup>3</sup> says one must guard against muscular resistance, for this causes increased blood-pressure. He compares his cases with those of normal persons and finds among the latter, variations in blood-pressure

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2. Pilez: *Wein. klin. Wchnschr.*, 1900, xii.

3. Kramer: *München. med. Wchnschr.*, 1892, No. 6.

between 90 and 180, but thinks these normal persons are not practicable subjects for comparison, as they are subject to various conditions which influence the pressure. He also insists that to arrive at a correct result one must take readings which cover a number of years. He calls attention to the fact that, in many instances, recovering patients take on flesh, and as this bodily change influences blood-pressure, a comparison of the readings taken before and after recovery in such a patient, or comparison of the readings in one case in which symptoms are present with readings in another case taken after recovery, are inaccurate. Although muscular action influences pressure in both anxious states and depressed phases of manic depressive insanity, yet aside from this factor there is a high blood-pressure because of the condition of mental depression, and when this mood changes there follows a change in blood-pressure.

I have made several tracings of blood-pressure in manic, agitated and depressed states, but will give only selected ones from each group. Of the manic phase of manic-depressive insanity, there are ten cases. The number of observations taken in this group varies from one to five in each case, and in instances of more than one reading, observations were taken a week apart. These cases are in part typical, the patients showing psychomotor restlessness and press of speech; other patients were merely stimulative, non-productive and irascible, while others were recovering or recovered. Of the depressed phase of manic-depressive insanity, there are six cases. In some of these cases six readings were taken. One of the patients grew mildly agitated during the taking of the reading and a few were stuporous. In the involutional group there are a number of patients — all over 50 years of age; one 73 years of age. A number of readings here were made in each case. Some of the patients have apparently lost affect, in that they pace the floor and wring the hands less, and they remain clear in mind although delusional and apprehensive. One patient is rather anxious and has motor agitation. While I realize that the cases are too few to warrant making any positive deductions from them, I find so many exceptions to the accepted dictum that I cannot entirely, if at all, agree with it.

In looking over the tracings taken in the manic cases, I was impressed with certain particular features which follow:

1. These tracings without exception show a curve of great amplitude (Fig. 1), more marked in the cases of psychomotor restlessness, but sufficiently demonstrable even in the non-productive cases to distinguish them from tracings obtained in cases from the other groups.

2. After the needle has begun to oscillate it rapidly jumps into full amplitude and the systolic blood-pressure is at once reached. This too is more marked the more evident the restlessness. In the other groups this period of oscillation before reaching systolic pressure is long drawn out.

3. After having begun to oscillate the needle works rapidly to the finish, indicating a rapid pulse.

4. The blood-pressure is raised and the pulse-pressure likewise is high.

Some of the cases selected in this group have arteriosclerosis; some have no sclerosis, at least demonstrable. I find in the former the same sort of curve as in the latter, though the sclerosis is a factor in raising the blood and pulse-pressure still higher in such cases of mania. I arranged in groups the patients in whom arteriosclerosis was and was not present and found the average blood-pressure and pulse-pressure higher in cases of mania in which there was arteriosclerosis than in cases of mania in which there was no arteriosclerosis. One sees in manic cases heightened color, sparkling eyes and a motor adjustment to a general feeling of exhilaration, and it is quite probable that in these tracings there is evidence of altered vasomotor tonus.



Fig. 1.—Pulse-tracing, Case 2. Note large amplitude, early onset and rapid action characteristic of tracings in manic states.

#### MANIC STATES

I shall present three cases which have both manic symptoms and arteriosclerosis.

#### REPORT OF CASES

CASE 1.—H., aged 63, has a history of previous attacks; is an exhibitionist; is sleepless, restless and has arteriosclerosis. The systolic pressure is 190 mm., the diastolic pressure 124 and the pulse-pressure 66. In a tracing taken in this case what has been observed as the special feature of the tracing in manic states is shown, namely, high amplitude, early onset of the full oscillation and rapid action. When the length of time that elapses before systolic pressure is reached is compared to the length of time that elapses before the same pressure occurs in depressed cases, there is found in the former only a few seconds, while in the latter as many as thirty seconds elapse.

CASE 2.—G., aged 43, has an attack of a few months' duration; has had several attacks; is irritable, vicious and has slight arteriosclerosis. The systolic pressure is 170, diastolic pressure 98, pulse-pressure 72 in one reading and again systolic pressure 144, diastolic pressure 92 and pulse-pressure 52 in another. The features again observed are large amplitude, early onset and rapid action (Fig. 1). Notice that the pressure varies. We cannot determine whether this

difference is due to the variance in the arteriosclerotic factor or to the manic factor. It was with great difficulty I could catch the systolic pressure because of its early onset after the beginning of oscillation.

CASE 3.—S., aged 43, has had a previous attack; is expansive, talkative and alert. The mania is of a religious type. The patient has arteriosclerosis. Five readings show:

Systolic pressure	Diastolic pressure
150	104
150	100
160	100
148	90
160	100

I had difficulty in catching the systolic pressure, so rapid was the onset of full amplitude.

These tracings do not vary from the others previously cited. The next four cases of mania have no arteriosclerosis. All are young individuals.

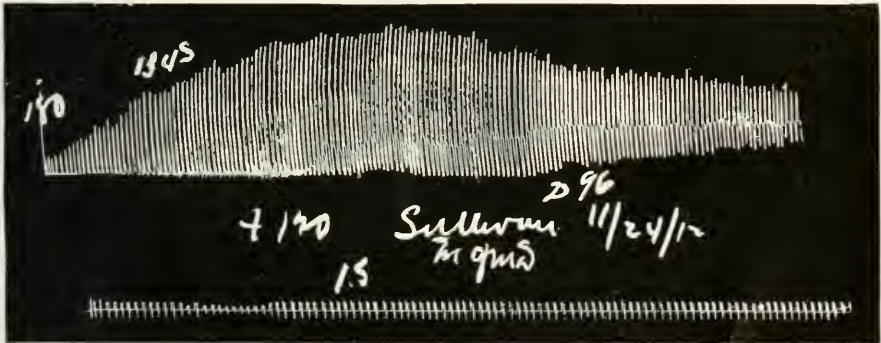


Fig. 2.—Pulse-tracing, Case 5. A typical tracing in manic cases

CASE 4.—S., a young woman, is excited, restless, talkative and distractible, but varies in the disturbed condition. Four readings are given:

Systolic pressure	Diastolic pressure
118	78
112	74
130	80
120	80

The first and second readings taken were lower. The first was taken during a very quiet period and the second followed a sedative pack. It would seem to indicate in the first instance a decrease in all the features of the manic tracing as restlessness diminished. In the second instance the sedative pack relaxed the general muscular tone. The other two tracings taken in this case show all the previously described features.

CASE 5.—S., a young man, has had a previous attack; is flighty, talkative and combative; physically, negative. The systolic pressure is 154, diastolic pressure 96 (Fig. 2). This is one of the most typical tracings I have illustrating manic features.



CASE 6.—T., aged 34, has mania of three months' duration. The patient has had a previous attack; is talkative, quarrelsome, expansive and irritable. The condition physically is negative. The systolic pressure is 160, diastolic pressure 108.

CASE 7.—O., aged 24, has had a previous attack of mania. The patient formerly had pressure of speech and activity, but now is emaciated; anemic, has a poor appetite and otherwise is negative physically. At present much less activity is shown. The large amplitude of the wave and early onset of the oscillation were present in the tracing, although the systolic pressure was 138 and the diastolic pressure was 80. The pulse-pressure was 58—not so high as in the more active cases, yet higher than normal and much higher than the pressure was in any of our stuporous cases or in the catatonic who sat next to him.

#### NON-PRODUCTIVE MANIAS

CASE 8.—W., aged 27, has mania of one and one-half years' duration. The patient is irritable but careless; is interested and happy but non-productive. There are no physical findings. Four readings are given:

Systolic pressure	Diastolic pressure
104	78
100	80
134	82
110	74



Fig. 3.—Pulse-tracing, Case 9. Persistence of characteristic features of manic states, though less marked in this case.

In one reading the patient was a little active, objecting to the procedure, and the systolic blood-pressure rose to 134. Otherwise the blood-pressure is near normal, although we still find the tendency toward larger amplitude, rapid onset and rapid action in the tracing, all of which were more noticeable at the time she was disturbed.

#### INTERMISSION IN A CASE OF MANIA

CASE 9.—McA., a young girl, who has recently been excited, is now quiet but not well. The systolic pressure is 130, diastolic pressure 90, pulse-pressure 40, all with a tendency toward a slight rise. The amplitude and other distinguishing features are noticeable (Fig. 3) but are not marked.

#### RECOVERY FROM MANIC STATE

CASE 10.—K., aged 30, has mania of one year's duration. She is negative physically; is talkative, flighty and active. Three readings are given:

Systolic pressure	Diastolic pressure
130	80
120	80
120	80

As she recovered the manic features in the tracing subsided and the blood-pressure dropped 10 mm.

## STUPOROUS CASES

Out of the stuporous cases I have selected two in which there are practically the same age and duration, the same degree of emaciation and the same mental manifestations. They have been selected for their similarity and also for their dissimilarity, in that one patient had arteriosclerosis and kidney lesion, while the other was free from these conditions.

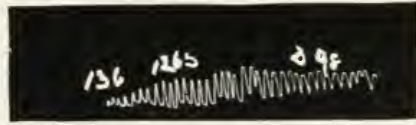


Fig. 4.—Pulse-tracing, Case 11. Small amplitude, slow action. Blood-pressure not above normal.

CASE 11.—N. J., aged 35, is married and has had a psychosis of one and one-half years' duration: is cyanotic, emaciated, stupid, retarded and depressed. There is slight arteriosclerosis. The urine shows a faint trace of albumin. Five readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
100	80	20
110	74	36
110	72	38
126	98	28
118	88	30

The average systolic pressure is 113, the average diastolic pressure is 83, the average pulse-pressure is 30.

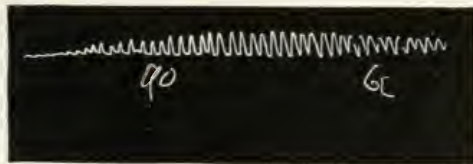


Fig. 5.—Pulse-tracing, Case 12. Small amplitude, slow action, low blood-pressure.

CASE 12.—A. F., single, aged 37. Psychosis of one year's duration. Ideas of unworthiness and commission of unpardonable sins are present. The patient is retarded, cyanotic and emaciated. Six readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
90	60	30
90	60	30
78	64	14
90	64	26
92	58	34
82	60	22

The average systolic pressure is 87, diastolic pressure 62, pulse-pressure 26.

In the case with the arteriosclerosis and kidney lesion (Fig. 4) the blood-pressure is higher than in the other case (Fig. 5). Both show small amplitude and slow action. The blood-pressure is quite low in the second case. In the first case it is not above normal.

## DEPRESSION CASES

CASE 13.—K., aged 54, a horse-shoer and a native of Illinois, has insanity of a few months' duration; is worried, hopeless, suicidal, depressed and slightly retarded. The heart and arteries are negative in condition. Albuminuria is present. Five readings are given:

Systolic pressure	Diastolic pressure
166	130
168	126
200	160
182	120
142	90

The pulse-pressure varies from 40 to 50.



Fig. 6.—Pulse-tracing, Case 14. Effect of muscular resistance.

The tracing which was taken in this case lacks the rapid onset seen in the tracing of the manic. The onset was more tedious and long drawn out. The amplitude varies as the blood-pressure varies, being greater in those tracings which exhibit the higher pressures. Here the factor seems to be the kidney lesion, which varies in its effect on the circulation. In no tracing made in these cases, however, is there shown the amplitude or rapidity seen in the manic case tracings. The mental condition seems to be the same from day to day. We will compare this case with one without arterial or kidney trouble.



Fig. 7.—Pulse-tracing, Case 15. Low amplitude, slow action and slow approach. Contrast with manic conditions.

CASE 14.—T., aged 34, has insanity of one year's duration. He has had previous attacks; is retarded, fearful, remorseful and subject to spells of agitation. The systolic pressure is 130, diastolic pressure 78. At the beginning of the experiment he was cooperative but became resistive and agitated, and with this the diastole became prolonged. This gives an increase in pulse-pressure, though the blood-pressure is low. The approach of the needle to the systolic reading is slow, the amplitude is small. This tracing (Fig. 6) also illustrates the effect of muscular resistance on the readings.

CASE 15.—D., aged 40, is depressed, hopeless, slightly retarded and working. The physical condition is negative. The average systolic pressure is 117, diastolic pressure 86, pulse-pressure 31. The blood-pressure is low, as is also the pulse-pressure. The same kind of tracing (Fig. 7) was obtained as in other cases of the same type, namely, low amplitude, slow action and slow approach. This is to be contrasted with the features in the previously described tracing in manic cases.



Fig. 8.—Pulse-tracing. Case 20, showing blood-pressure rise with muscular resistance.

CASE 16.—B., aged 29, has a first attack of insanity of a few months' duration. Ideas of unworthiness and a feeling of insufficiency are present. The patient is in a negative physical condition. The systolic pressure is 106, diastolic pressure 78, pulse-pressure 28. Low pressure, both of blood and pulse, and the same kind of tracing as previously described, were noted.

#### MELANCHOLIAS

In these cases is seen (Figs. 8 and 9) the effect of muscular resistance in raising the blood-pressure regardless of the condition of the arteries. After such patients become calm the blood-pressure falls nearly to normal if there is no arterial disease, and while the mental state is constant, the



Fig. 9.—Pulse-tracing, Case 20, showing blood-pressure rise with muscular resistance.

blood-pressure varies, depending more on the variation of peripheral resistance than on any change in mood. These patients are in middle life and a higher average might be expected, other conditions being normal.

In those cases in which arteriosclerosis is present the average blood and pulse-pressure are higher than in the cases in which arteriosclerosis does not exist.



CASE 17.—C., aged 48, has melancholia of three years' duration. The patient had a previous attack; is poorly nourished, tuberculous, agitated at times, deteriorated, depressed and stupid; a marked arteriosclerosis exists.

This case really belongs to those classified as stuporous depressions, though the blood-pressure and pulse-pressure were slightly high for a stuporous case. Four readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
110	86	24
114	84	30
126	88	38
132	90	42

This could be accounted for by the arteriosclerosis. The amplitude of the wave was a little larger on this account, but far short of the large amplitudes seen in the tracings of manic cases.

CASE 18.—C-2, aged 56, has melancholia of a duration of one year. The patient has ideas of persecution and hallucinations. Though at first she was agitated, restless, and apprehensive there was later a deterioration to fussing, upbraiding and crying, though the condition was one of tolerance. Five readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
136	88	48
112	80	32
110	80	30
130	90	40
120	88	32

In this case the tracings show moderately low blood-pressure and pulse-pressure which do not rise except when the patient resists, which occurred when the readings were taking. Nothing distinctive was shown in these tracings.

CASE 19.—S., aged 55, has melancholia of two years' duration; has delusions of persecution, is suicidal, distressed and has periodically marked anxiety. The physical condition is negative. Seven readings are shown:

Systolic pressure	Diastolic pressure	Pulse-pressure
130	70	60
110	70	40
112	70	42
120	70	50
120	74	46
130	80	50
120	90	30

The first and sixth readings in which the blood- and pulse-pressure are 130 and 60, 130 and 50, respectively, show the effect of her resistance. This does not reach the height seen in tracings of manic or arteriosclerotic cases, and distinctly lacks the special features noted in the tracings of the former.

CASE 20.—M., aged 45, who was formerly suicidal and anxious, is now empty and mildly resistive at times. A slight arteriosclerosis exists. Six readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
160	100	60
116	80	36
134	80	54
122	88	34
140	90	50
110	76	34

The first reading shows the effect of muscular resistance in raising blood-pressure and pulse-pressure (Figs. 8 and 9).

CASE 21.—M., aged 73. is agitated, unclear: has feeling of unworthiness, is excited and apprehensive. The ideas are all present but the affect is gone. Arteriosclerosis exists. Six readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
160	80	80
140	80	60
170	70	100
140	88	52
130	80	50
160	80	80

Though the mental condition did not change, the blood-pressure did, owing to the variation in the peripheral resistance in the sclerosed arteries. This shows the value of many readings in any case. One reading was 170, another 130 for systolic blood-pressure.

CASE 22.—B., has melancholia of one and one-half years' duration: is depressed, has feeling of unworthiness, is apprehensive and suicidal. The arteries are palpable. Deteriorated to an empty condition. Four readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
160	100	60
140	110	30
146	102	44
160	110	50

The patient was neither agitated nor resistive. The high blood- and pulse-pressures are due to the sclerosis of the arteries.

#### CONCLUSIONS

1. Cases of mania show typical features in the tracings which are taken, and these are probably due to an altered state of vasomotor tonicity in keeping with the other motor phenomena in these cases. These features are large amplitude of the curve, rapid action and short interval between beginning of oscillation and full amplitude. The blood and pulse-pressures are raised and are more marked, as are the other features just spoken of, as the restlessness becomes more marked. All decrease as the patients recover. In non-productive cases these features are much diminished, but still demonstrable. Arteriosclerosis existing in manics raises the blood- and pulse-pressures still higher. The usual features of the manic tracing are as apparent here as in cases without arteriosclerosis, and the larger amplitude, rapid action and early onset in the tracing are not features of the sclerosis, but of the manic state. In cases of sclerosis occurring in other types of psychosis these features are lacking.

2. In stuporous cases the blood- and pulse-pressure are quite low. The amplitude is quite small and action is slow and tedious. This seems to indicate a condition of relaxation of the blood-vessels, in keeping with the other phenomena exhibited.

3. In depressed cases the blood- and pulse-pressures are lower than in the manic states, but higher than in the stuporous states. The amplitude of the wave, its onset and rapidity is less than in the manic states, but higher than in the stuporous states. Any rise in any of these things is

due either to muscular resistance or physical disease and not to any state of mind.

4. In melancholia the average blood-pressure is relatively high, for the patients are among those in middle life. The blood-pressure and pulse-pressure are near normal as long as there is no muscular resistance, and no other factor, such as arteriosclerosis, to produce a rise. The tracings show nothing peculiar, except when these factors are added, and then blood-pressure is raised; the amplitude of the wave is also slightly increased.

5. By taking many readings it may be possible to attribute high blood- and pulse-pressures in some cases to the varying effect of arterial and kidney disease on peripheral resistance rather than to the effect of the mental condition. In other cases resistance on the part of the patient is a factor in raising the pressure — a fact which might be overlooked had only one reading been taken at such a time.

## AGE INCIDENCE IN CARCINOMA \*

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The progressive increase in the incidence of carcinoma as the middle period of life is approached and reached has long been recognized. Most writers have contented themselves with a statement of this fact, leaving the reader to understand that even in advanced years the progressive increase is maintained. For instance, Bashford<sup>1</sup> says: "Carcinoma appears in the human subject with increasing frequency as life advances." He recognizes, however, that the carcinoma incidence in those organs which undergo a definite phase of involution long before senile changes in the organism as a whole are marked, is highest during such involutionary processes. This he bases on statistics of breast and uterine cancer.

Senn<sup>2</sup> gives a suggestion that in extreme old age the cancer incidence is decreased, as the following quotation shows: "Carcinoma manifests a predilection for the conditions incident to senile marasmus, occurring most frequently in persons between 50 and 70 years of age."

It was in the hope of throwing additional light on this and allied problems that the analysis here presented of over 1,100 microscopically verified cases of carcinoma was undertaken.

Several varieties of cancer statistics have been made use of in similar investigations. Such are mortality returns, cancer censuses and hospital and laboratory reports. From the standpoint of a study of age-incidence the first-named fails, in that mortality returns give only the age of the victim when the disease has overpowered him; the cancer census fails in that the report is encumbered with unverified cases; while the greatest objection that can be raised against hospital and laboratory reports is that they are not derived from a representative fraction of the total population.

Our material consists of consecutive cases of carcinoma of known age, diagnosed in the Pathological Laboratory of the University of Michigan between the years 1895 and 1913. This material possesses the following advantages: 1. Every case has been microscopically verified. 2. The age of the patient is obtained at a much earlier stage than in mortality records and thus the actual age-incidence is more nearly represented. 3. The cases are derived from a fairly representative fraction of the total population. The larger part are from the university hospitals, which receive

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\* From the Department of Pathology, University of Michigan.

\* Submitted for publication August 8.

1. Bashford: *Imp. Cancer Research Fund*, 1905, No. 2, p. 32.

2. Senn: *Pathology and Surgical Treatment of Tumors*, p. 66.



patients from the entire state, and, to a lesser degree, from neighboring states. Because of this fact, and also because they are not charity hospitals, the patients are much more representative of the general population than is ordinarily the case.

The figures for the age distribution of the total population are derived from the United States census report for 1900.<sup>3</sup> This census report was chosen as being more nearly at the midpoint of the period during which the cases occurred than was the census report of 1910. Since more than 90 per cent. of the cases reported are from Michigan, the figures for that state alone were considered. The error here is negligible for the varia-

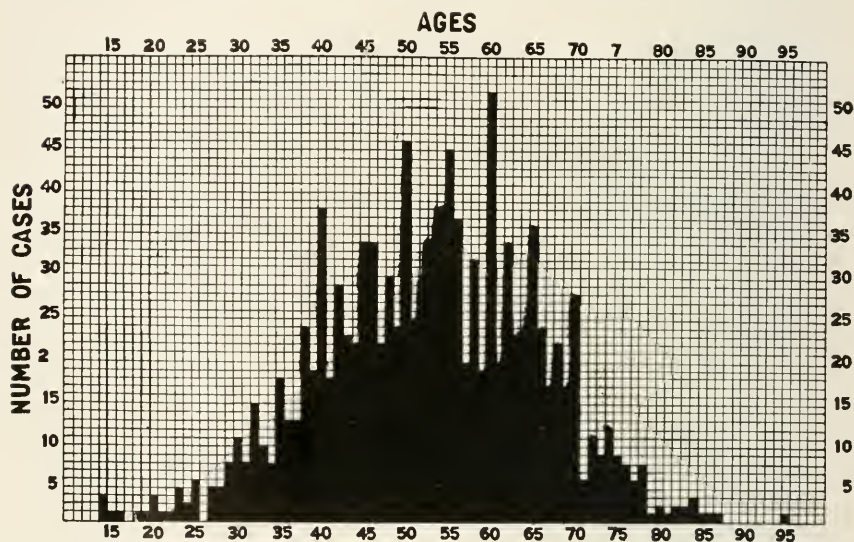


Chart 1

tions in age distribution over a period of a few years, or in neighboring states as compared to Michigan in 1900, are very slight.

The yearly distribution of the total 1,106 cases is shown by Chart 1. The greatest number for any year is found at the age of 60 with fifty-one cases. The error of approximate answers is well shown by the disproportion in the number of cases for consecutive years. The multiples of five are favored by patients in giving their ages. The even ages show a slight preponderance over the odd. This disproportion is found in some degree in all census statistics.<sup>4</sup> In order to avoid this source of error all analyses have been based on five-year periods having the multiple of five as the middle year in the group. Thus the age period 58 to 62 will presumably represent an accurate grouping of all of the cases given as of age 60,

3. Report, Twelfth Census, U. S., 1900, ii, Part 2, 52.

4. Report, Twelfth Census, U. S., 1900, ii, Part 2, 35.

while if the usual quinquennial groups were used, the group 60 to 64 would include many cases which in reality belong in the preceding period.

The percentage of the total population living during each age period and the percentage of the total number of cases of carcinoma for each age period are shown by the two curves of Chart 2. The carcinoma curve lies below the population curve up to age 37. Here the curves cross. At this point the ratio between the two curves is unity, and it may be interpreted as being the point at which the number of cases of carcinoma is the same as it would be were carcinoma uniformly distributed throughout the total population. Beyond this point the ascent of the curve of

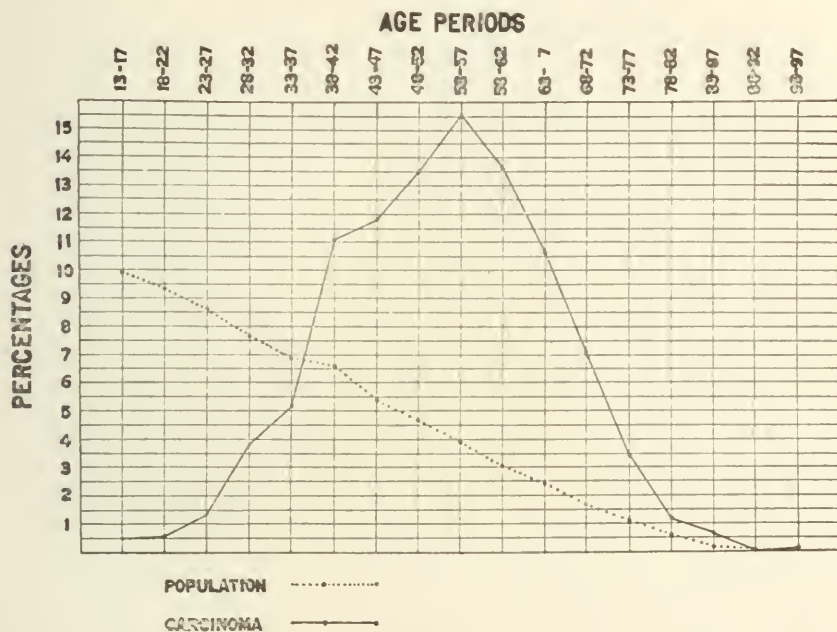


Chart 2

carcinoma percentages is very rapid and the highest point is reached in the age period 53 to 57.

This does not mean that the carcinoma incidence is greatest during this age period, for the decreasing population leaves fewer individuals to be attacked. This point has been further elucidated by computing a series of ratios between the percentages previously used, that is,

$$\text{Ratio for age period } N = \frac{\text{percentage of carcinoma for age period } N}{\text{percentage of population for age period } N}$$

These ratios are plotted in the curve of Chart 3. As previously mentioned, the value is less than one up to age 37 and greater than one thereafter. The apex of the curve is at the age period 58 to 62, and this, therefore, represents the age period of greatest carcinoma incidence.

From 58 to 62 there is a definite fall in the curve, which becomes most marked after 66 to 72. The abrupt rise after 78 to 82 is meaningless, since it is determined by too small a number of cases (eight) to have any value. One must therefore conclude that after 70 years of age there is a decided diminution in the incidence of carcinoma.

Chart 4 shows graphically the age distribution of our cases of carcinoma divided according to sex. The cases in males totaled 495; in females 611. It is noticeable that the curve for female cases rises on the average ten years before that of males, reaches its apex ten years earlier and falls on the average five years ahead of the curve for males. Since,

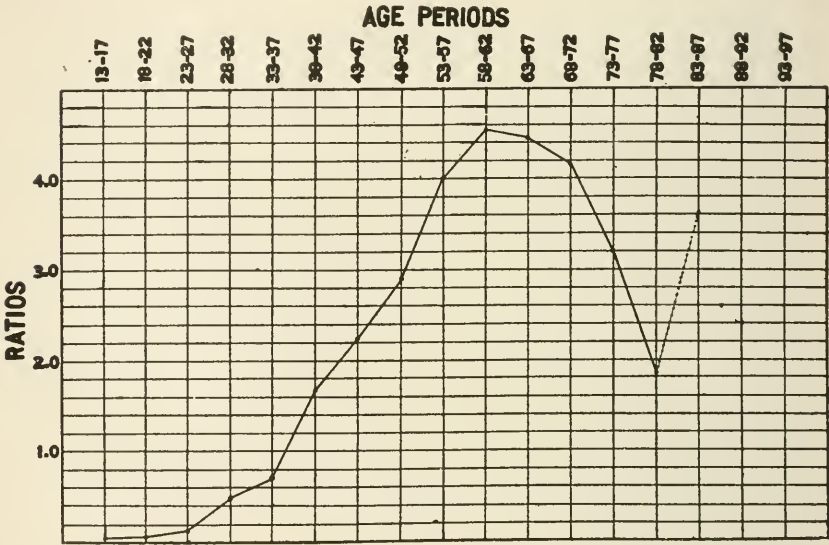


Chart 3

however, the total number of cases differs with the sexes, and the population totals for the sexes are also unlike in their age distribution, no definite conclusions can be drawn from the data presented by Chart 4. It has, therefore, been necessary to construct ratios on the same general plan as before, that is,

Ratio for males, age period N =  $\frac{\text{percentage of carcinoma in males at age period N}}{\text{percentage of male population at age period N}}$   
and

Ratio for females, age period N =  $\frac{\text{percentage of carcinoma in females at age period N}}{\text{percentage of female population at age period N}}$

By plotting the two series of ratios a graphic representation of the incidence of carcinoma in each sex and at each age period is obtained and a direct comparison is possible (Chart 5). The ratio for females reaches unity about the age 33, while for males this point is not reached until age 39. Among females the incidence of carcinoma reaches its height in

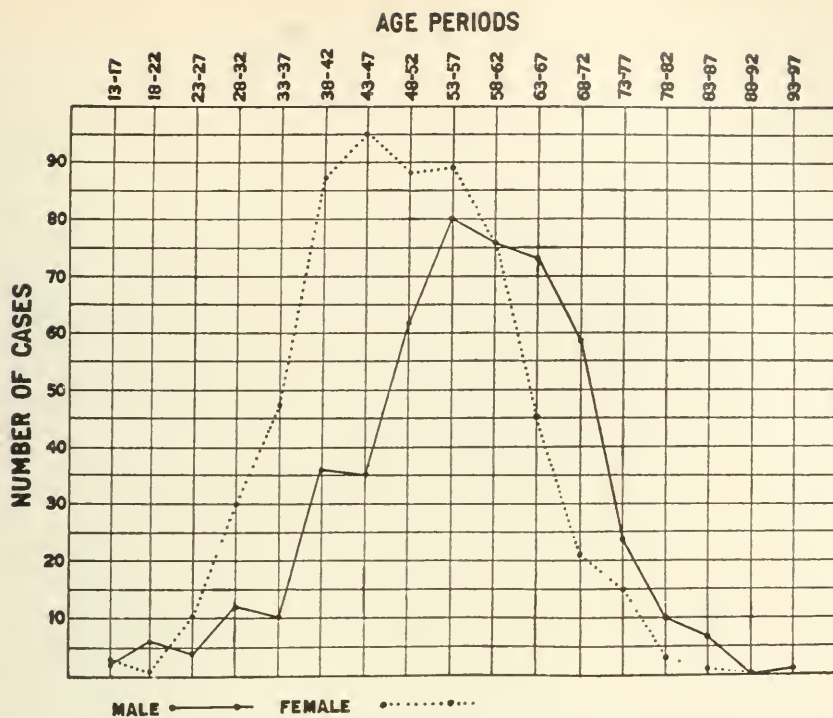


Chart 4

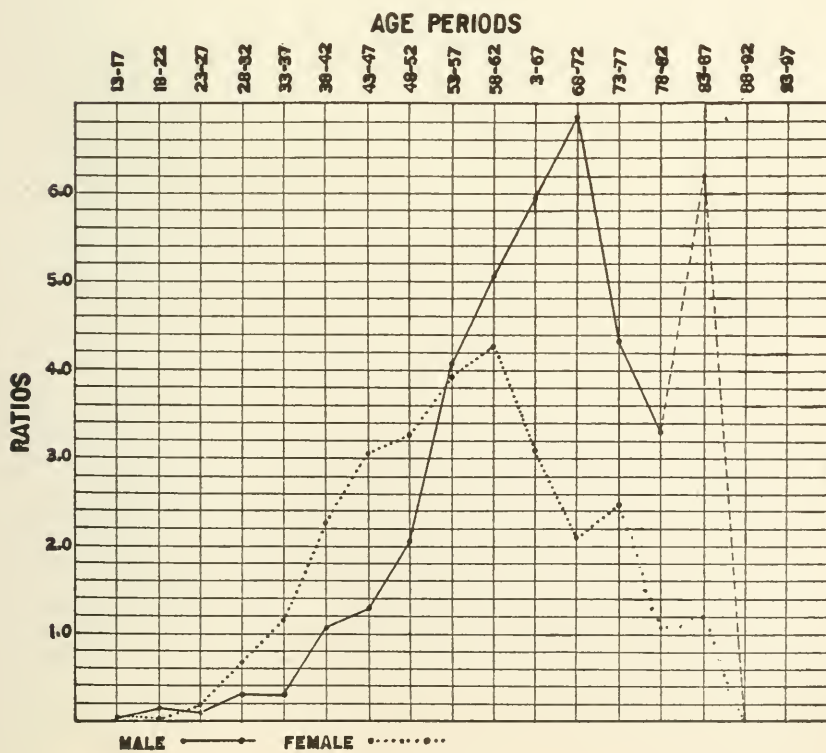


Chart 5



the age period 58 to 62, while carcinoma is most frequently recognized in males at 68 to 72. These latter figures are made somewhat higher than they should be by the cases of basal-celled carcinoma which, occurring most frequently in males, are often diagnosed rather late in life, though they may have been of many years standing. But, even with due allowance for this source of error, it is evident that in the female the wave of carcinoma incidence traverses its cycle five to ten years earlier than in males. The sharp decrease in both curves after the crest of the curve is reached holds good for each sex quite as well as it did in the combined statistics of Chart 3.

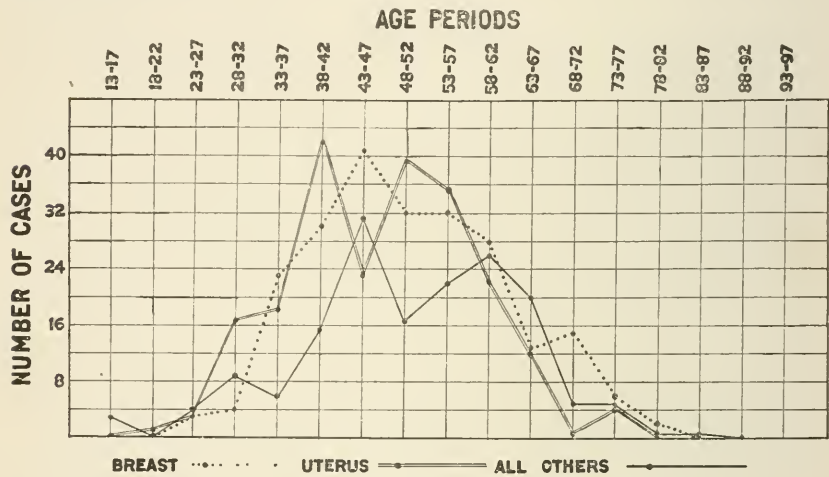


Chart 6

In order to discover what elements in the curve of female cases of carcinoma were responsible for causing it to precede the male curve in all its phases, the female cases were divided into three groups according to the location of the carcinoma.

Breast .....	229 cases.
Uterus .....	219 cases.
All others .....	165 cases.

The age distribution of each of these three types is shown by Chart 6, and in Chart 7 the percentages of the total number of cases in each group occurring during each age period are plotted. It is found that between 33 and 43 the percentages of cases in which carcinoma of the breast and uterus occurs are much higher than that of the group which is designated "all others." These two sources, comprising more than two-thirds of all our cases of carcinoma in women, are therefore very powerful factors in determining the earlier cycle in the general curve for women.

The form of the curves in this chart becomes less regular because of the smaller number of cases being considered. There is one irregularity,

however, which while it may be due to the smaller number of cases, may also have a much deeper significance. In the curves which indicate cases in which carcinoma of the uterus occurs, we find:

42 cases, or 19.4 per cent., at age period 38 to 42  
 23 cases, or 10.6 per cent., at age period 43 to 47.  
 39 cases, or 18.0 per cent., at age period 48 to 52.

The peculiar drop just at the menopause period, 43 to 47, may find its explanation in the fact that metrorrhagia at this time is usually considered by the patient as but a phenomenon of the climacteric, and therefore unworthy to be investigated.

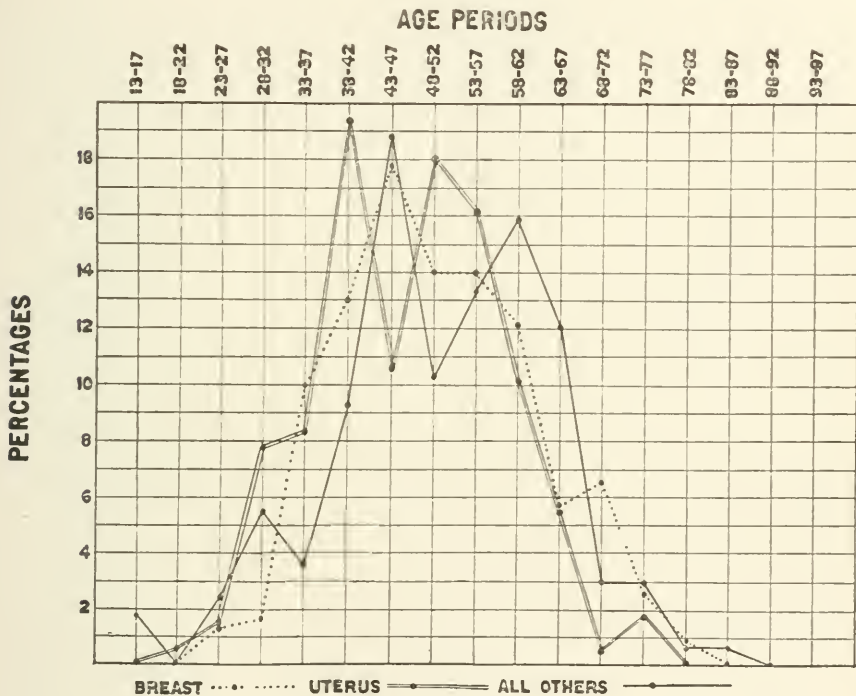


Chart 7

#### CONCLUSIONS

The analysis here given indicates that:

1. The incidence of carcinoma is greatest at the age period 58 to 62.
2. After this period the carcinoma incidence decreases.
3. Carcinoma incidence in women runs a definite cycle which is roughly parallel to that in men, but precedes it by five to ten years.
4. The earlier age for the phases of the curve for women is largely due to the earlier appearance of carcinoma of the breast and uterus as compared to other varieties of carcinoma.

## HEREDITY WITH REFERENCE TO CARCINOMA

AS SHOWN BY THE STUDY OF THE CASES EXAMINED IN THE PATHOLOGICAL  
LABORATORY OF THE UNIVERSITY OF MICHIGAN,  
1895-1913 \*

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The statistical study of carcinoma is regarded by many writers as having been carried as far as it can be profitable; and certainly but little that is new has been gained through this method during the last decade. Nevertheless, its possibilities have not been exhausted; and it is highly desirable that the whole neoplasm problem in all of its aspects be attacked again from the statistical standpoint, though in a somewhat different way. Practically all of the old statistical studies of neoplasms, particularly those of carcinoma, were based on mortality reports; or if not on these, on morbidity reports based on clinical diagnoses. In very few instances only has the statistical study been carried out on the basis of the records of a diagnostic pathological laboratory. Statistics of neoplasm from such a source must be of infinitely greater value than those founded on mortality statistics. In the records of the diagnostic laboratory the diagnosis is based on the histological examination, and the percentage of error is reduced to a minimum. In the mortality statistics, on the other hand, the diagnoses are chiefly clinical, and consequently subject to the wide error inherent in the clinical diagnosis of "tumor," neoplasm, "cancer" and the like. Moreover, the material coming to the diagnostic laboratory is usually seen from two to five years earlier than the mortality age. In studies relating to the age-incidence of any form of neoplasm it is evident that the records of the pathological laboratory for that neoplasm will be much more trustworthy than the mortality statistics. It is also possible many times in the diagnostic laboratory to follow the course of a neoplasm over a definite period, so that important practical knowledge may be gained as to rate of growth, recurrence, healing, metastases, etc.

The following study of the influence of heredity on carcinoma is taken from the records of the pathological laboratory of the University of Michigan during the years of my service from 1895 to 1913. During this period 3,600 cases of neoplasm of all varieties have been studied, either in material taken for practical diagnosis or obtained by necropsy. Of these 3,600 cases, some 1,600 were cases of carcinoma, as was shown by the microscopic diagnosis. Practically every variety of carcinoma

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\* Submitted for publication Aug. 8, 1913.

described in the literature, and a few others, as well, are to be found in this material; and the same is true of the other forms of neoplasm. While carcinomas of the breast, uterus and lip form the greater part of the cases examined, all other localizations are represented. Another great advantage is that about 90 per cent. of the material was derived from the general population of the state of Michigan. The fact that the university hospital is a state hospital and not a charity institution, gives it a much more representative population than would be found in the charity hospitals of the greater cities.

The difficulty of obtaining a family history from patients in charity hospitals is well known. Few of them know the cause of death in their grandparents or in other members of that generation. The same thing is true, though to a less extent, of the patients from whom the material used in this study was obtained. Therefore what worth this study may have must be positive; the negative findings cannot be taken as absolute. In about six hundred cases of carcinoma no family history was obtainable; in the majority of those in which a family history was obtainable the details are meager. In spite of these handicaps, in a surprisingly large number of cases a family history of carcinoma is given — about 15 per cent. of all in which the family history is obtainable.

The existence of a family susceptibility to carcinoma, denied by many writers, affirmed by others, has again become, as a result of the studies of endemic carcinoma in mice, a question exciting lively interest. These investigations appear to show that certain family strains in mice do not develop spontaneous tumors, and that, further, such strains are resistant to tumor transplantation from other mice. Other family strains show a high frequency of spontaneous tumors, and in such strains transplantation may be successfully carried out. It would appear then, that the latter strains possess a familial predisposition or susceptibility to the development of neoplasm, and that intrinsic or inherited factors play some part in the development of neoplasms. Bashford denies this, and Tyzzer, who carried out breeding experiments with animals representing different strains, was unable to find that the transmission of the predisposition to neoplasm followed the laws of heredity.

On the other hand, a family predisposition in the human species to certain forms of neoplasm has long been recognized, particularly in the case of certain benign neoplasms (fibroma, lipoma, chondroma, osteoma, angioma, leiomyofibroma, glioma, neurofibroma, papilloma, adenoma and cyst-adenoma), and to a less marked degree in the case of certain forms of sarcoma and carcinoma. While the existence of such a predisposition is generally accepted by text-books on pathology, no good statistical studies exist of the hereditary occurrence of these neoplasms.



Cancer surveys of living patients, as conducted in Germany some years ago, have given little information concerning the family occurrence of neoplasms, chiefly for the reason that it is practically impossible to trace the members of a given family through more than one or two generations, and unless all of the members of the family for several generations can be considered, no accurate conclusions can be drawn. The difficulties attendant on such a statistical study are very great; and except in rare cases complete family records of cancer incidence are not found in the literature. One of the most striking is Broca's case,<sup>1</sup> also quoted by Levin, who criticizes the method of investigation, because no comparison was made between the number of the affected and unaffected members in each generation. If the total number of ascendants is not considered, and only those affected by carcinoma picked out, a family may appear to be a cancer family when in reality the proportion of cancerous to non-cancerous members is very small. As Levin states, Broca's family might give very different results if studied from the standpoint of the history of all its members.

Levin, to my knowledge, is the only one who has attempted a statistical study of heredity in cancer from the standpoint of an entire family history. He collected data<sup>2</sup> from five families, in two families fairly complete and in three fragmentary. In the families studied he noted that they were characterized on the whole by the occurrence of cancer of the intestines in the males, and cancer of the breast in the females. His charts show that the incidence of cancer in these families is not greater numerically than would be found in the population as a whole, but that the occurrence of "cancerous fraternities" (a fraternity in which one or more members suffer from cancer, with a history of cancer either on the maternal or paternal side, or both) speaks for some influence of heredity on cancer. In other words, a cancerous fraternity must mean the union of two germ-plasms, each of which is characterized by the presence of germ-cells that are non-resistant to cancer.

In my material of 3,600 cases of neoplasm examined pathologically, there were 1,600 cases of carcinoma. About one thousand of these gave fairly good family histories with the ages of the members. A smaller number (30 per cent.) gave detailed histories. From this number are taken the families which show multiple occurrence of carcinoma. In many of these all of the members of the family for three generations are given; in others, the records are incomplete. Four families give complete records of the descendants of the cancerous grandparent. The incidence of cancer in these families is so striking that it can be interpreted as showing an inherited susceptibility to cancer.

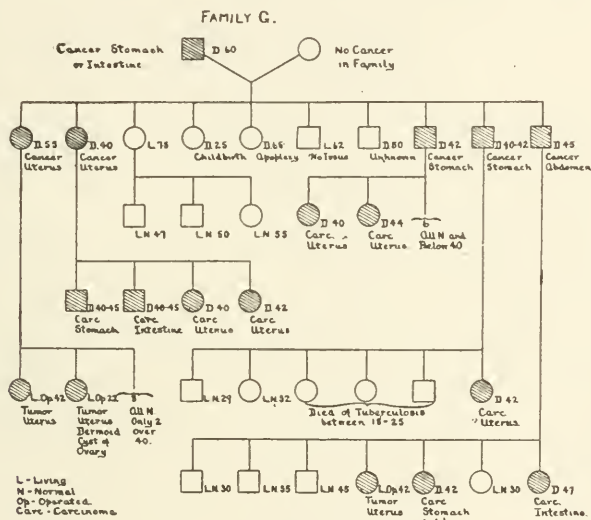
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1. Broca: Quoted by Wolf, *Die Lehre von den Krebskrankheiten*, Jena, 1907; Williams, p. 369.

2. Levin: *Ztschr. f. Krebsforsch.*, 1912, ix, S. A., p. 5.

## CANCER FAMILIES

FAMILY G. (Chart 1).—In this family a fairly complete survey was made of the two generations derived from a cancerous grandfather with a traditionary history of cancer in his line and a grandmother with a normal family history. From these there were ten children, five males and five females. Two of the daughters died of cancer of the uterus at 55 and 40 years, while two sons died at 42 of cancer of the stomach, and a third one at 45 of cancer of the abdomen. All five of these individuals were married to normal partners without a family history of cancer, and all had issue, as follows: Oldest daughter who died at 55 of cancer of the uterus, had ten children; one daughter operated on at 42 for "cancer" of the uterus and still living; another daughter operated on at 22 for uterine tumor and bilateral dermoids of ovary, and still living. The remaining eight children are all living and well, only two being over 40 years of age. The second daughter, who died at 40 of cancer of the uterus, had four children, two sons and two



daughters all dying of cancer, the two sons of cancer of the stomach and intestine, and the two daughters of carcinoma of the uterus. The third daughter, living and well at the age of 75, has three normal children living at the ages of 47, 50 and 55. Four children had no living issue. The eighth child, a son, died at 42 of cancer of the stomach. His wife was of normal family history. They had eight children, of whom two daughters have died of cancer of the uterus at 40 and 44 years, while the remaining six are all living and well below the age of 40. The ninth child, a son, died of cancer of the stomach when between 40 and 42 years of age. He left six children from a marriage contracted with a woman of non-cancerous family history. One daughter died at 42 of cancer of the uterus, three children died of tuberculosis between the ages of 18 and 25 years, while two others are living and well at the ages of 32 and 29 years. The tenth son died at 45 of cancer of the abdomen, most probably primary in the stomach. He left, from a marriage contracted with a woman of non-cancerous family, seven children, of whom one died at 42 of cancer of the stomach and liver, another at 47 of cancer of the intestine, while a third was operated on at 42 for tumor ("cancer") of the uterus, and still lives in apparent good health. Four others are living and normal at the ages of 45, 35, 30 and 30.

Of the forty-eight descendants of the cancerous grandfather seventeen have died or been operated on for "cancer." The preponderance of carcinoma of the uterus (ten cases) and of the stomach (seven cases) is very striking in the family history.

FAMILY F. (Chart 2).—In this family the maternal grandmother died of tumor. Her non-cancerous brother had two children, both of whom died of "cancer." Her only son died at 61 of dropsy. He married a woman who had two brothers who died of cancer of the stomach. She herself died of Bright's disease at 75. Her

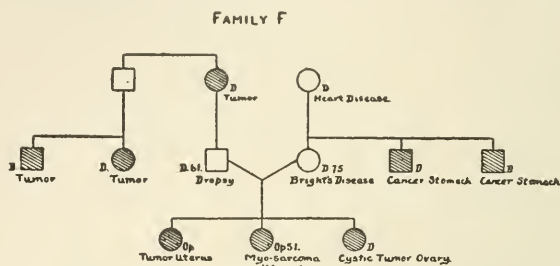


Chart 2

mother died of heart disease. The three daughters of this pair who show a double family history of susceptibility to cancer all had neoplasms; the oldest was operated on for tumor ("cancer") of the uterus and is still living; the second was operated on at 51 for myosarcoma of uterus, while the third daughter died of cystic tumor of the ovary. In this family history the preponderance of stomach and uterine neoplasms is also shown.

FAMILY P. (Chart 3).—The paternal grandfather had a nephew who died of cancer of the lip. In the first filial generation there was one daughter who died at 35 of cancer of the lip and a son who died at 86 of cancer of the scalp and cervical lymph-nodes. This son married a non-cancerous woman whose only sister

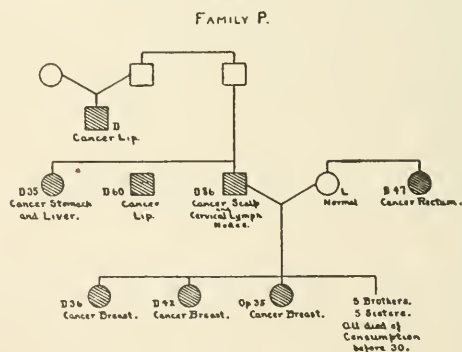


Chart 3

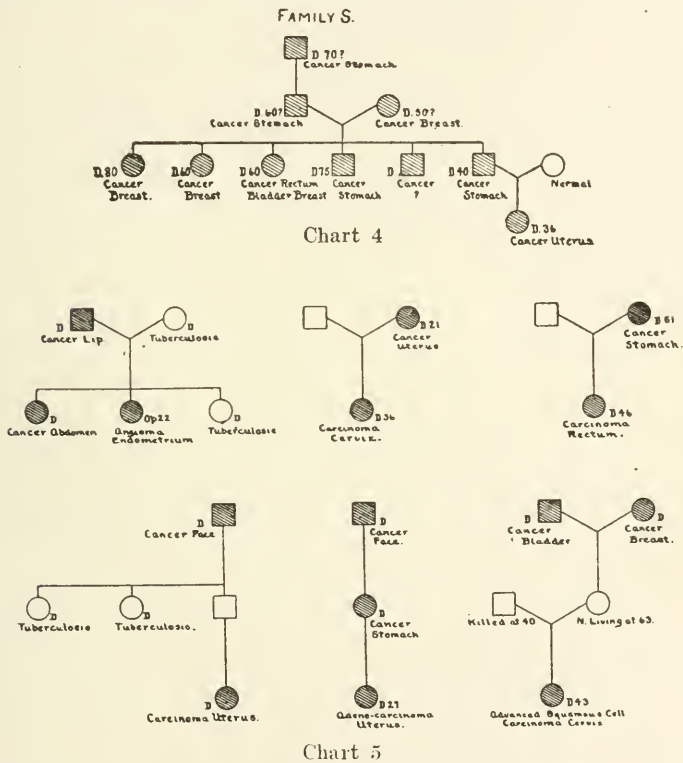
had died at 47 of cancer of the rectum. From this union thirteen children, ten of whom (five brothers and five sisters) all died of pulmonary tuberculosis before the age of 30, while three remaining daughters had carcinoma of the breast, two dying at the ages of 36 and 42; and one operated on at 35.

FAMILY S. (Chart 4).—The paternal great-grandfather died at about 70 of cancer of the stomach. His only son died at about 60 of cancer of the stomach, having married a woman who died at about 50 of cancer of the breast. They had six children, all of whom died of cancer: two daughters died at 80 and 60 of cancer of the breast, and another at 60 of multiple carcinoma of the breast, bladder and

rectum. Two sons died of cancer of the stomach at the ages of 75 and 40, the third son dying of cancer of some internal organ, most probably the stomach. Only one son had issue, by marriage with a normal line. The only child died at 36 of cancer of the uterus. Of the eight descendants of the cancerous great-grandfather all died of cancer. As in Family P., the occurrence of carcinoma in both paternal and maternal lines apparently strengthens the susceptibility, both families becoming extinct.

## CANCEROUS FRATERNITIES

These are of much more frequent occurrence in the case-histories of carcinoma (Charts 5, 6, 7, 8, 9) than the striking family histories given above show. The reasons for this are obvious: Few individuals of the



general run of the American population know anything about their family history except for the immediate members. By far the majority do not know the cause of death of their grandparents. Twenty-nine cancerous fraternities are selected as representative of our case-histories. Two generations only are represented in the majority, but in some of them three generations, and in one instance, four generations are shown. The normal members of the second and third generations are also given, so that the proportion of cancerous to non-cancerous individuals in two generations is exact. The majority of the cancerous fraternities occur in small families, and in many cases the patient from whom the material



examined came represented the end of the family line. In several instances all the members of the small family are cancerous. The charts explain themselves. The most striking thing shown, aside from the susceptibility of the family group, is the great prominence of tuberculosis

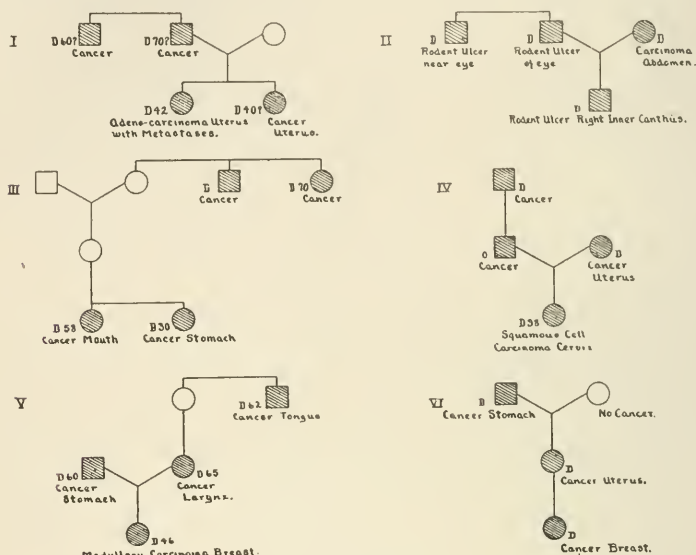


Chart 6

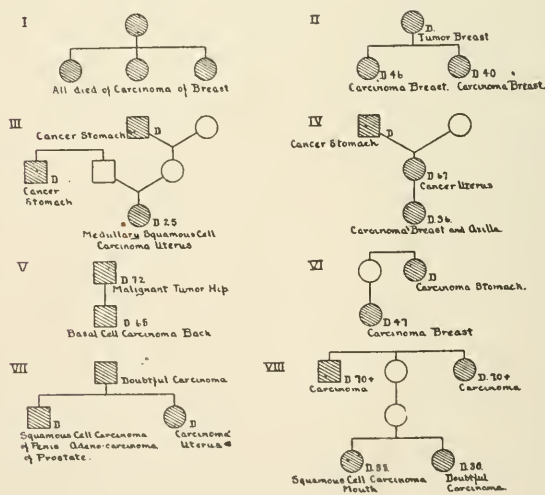


Chart 7

as the most commonly associated disease of the cancer stock. The two susceptibilities seem to run together, at least, in so far as our family histories are concerned. A tuberculous ancestry is not infrequently found in families where there is no family history of cancer back of the present

generation. Next to tuberculosis the diseases most commonly associated with carcinoma are cardiac and renal disease.

It will be noted that the uterus, breast, gastro-intestinal tract and mouth are the parts of the body most frequently involved in the case of these family cancers. Cancer of the lip and rodent ulcer of the face show also a tendency to family occurrence.

The study of a large number of cases of carcinoma yields isolated but striking examples of a marked family occurrence through several generations; and a much more frequent family group or "cancerous fraternity" occurrence. From such histories it is hardly possible to draw any other conclusion than that a definite cancer susceptibility exists in certain families. The great frequency of the association of cancer with tubercu-

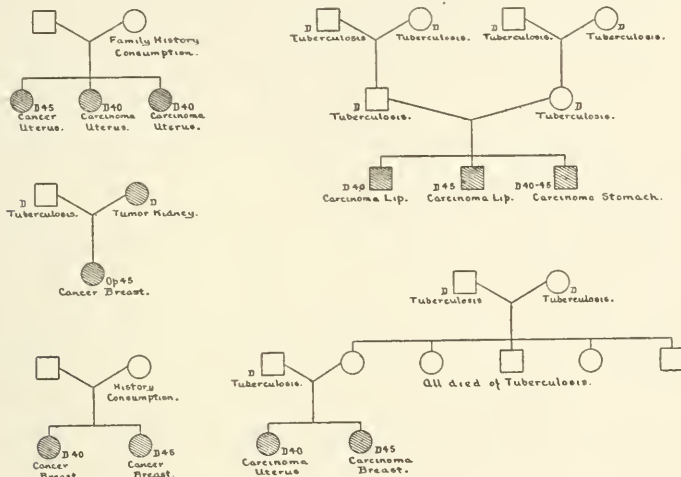


Chart 8

losis might be taken as an evidence of a general weakened resistance on the part of the family lines; and this conclusion is supported by the extinction of many of these lines through a lessened fertility.

In the study of all of our neoplasm material a family susceptibility is occasionally shown in the case of angioma, lymphangioma, fibroma, neurofibroma, lipoma, myofibroma of uterus, adenoma of breast and adenoma of thyroid; but extremely rarely in the case of sarcoma.

#### CONCLUSIONS

1. A marked susceptibility to carcinoma exists in the case of certain family generations and family groups.

2. This susceptibility is frequently associated with a marked susceptibility to tuberculosis, and also with reduced fertility.<sup>3</sup>

3. The striking association of tuberculosis with cancer in certain families has also been noted by Kuthy and Williams, "The Natural History of Cancer," p. 371.

3. The multiple occurrence of carcinoma in a family generation practically always means its occurrence in a preceding generation.

4. The family tendency is usually more marked when carcinoma occurs in both maternal and paternal lines.

5. Family susceptibility to carcinoma is shown particularly in the case of carcinoma of the mouth, lip, breast, stomach, intestines and uterus.

6. In a family showing the occurrence of carcinoma in several generations there is a decided tendency for the neoplasm to develop at an earlier age in the members of the youngest generations. In this case the neoplasm often shows an increased malignancy.

7. Because of the difficulty of obtaining complete family records, the laws of inheritance of carcinoma susceptibility cannot be determined

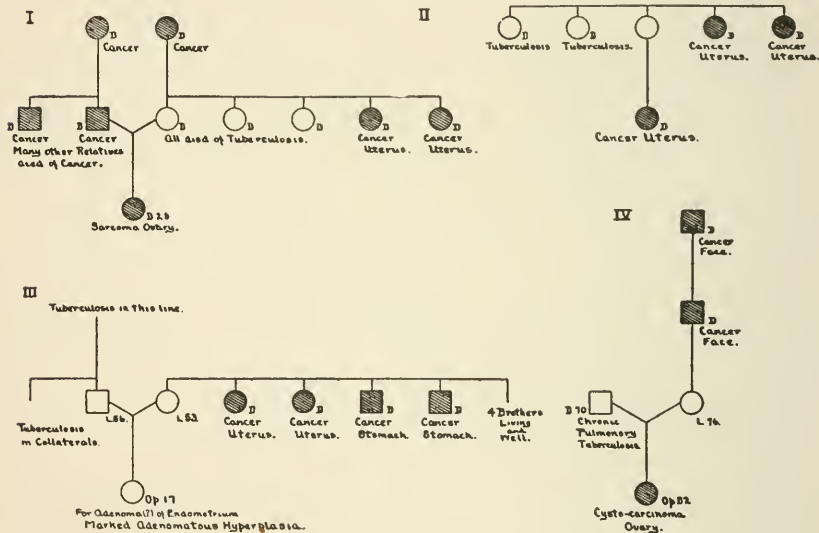


Chart 9

accurately, and it is highly desirable that investigations of large family records should be made relative to the occurrence of carcinoma susceptibility. In Levin's study of cancerous fraternities in connection with the whole family history the percentage of cancerous members in each cancerous fraternity corresponds very closely to the Mendelian percentage of members with recessive unit-characters in a hybrid generation. The same conclusion might be drawn from my cases in certain instances, but it does not seem to me that the data are sufficient for such conclusions. He himself does not consider this conclusion as final. Levin also concludes that resistance to cancer is a dominant character whose absence creates a susceptibility to cancer. While some of my cases show a family history suggesting this, others would indicate a progressive degenerative

inheritance—the running-out of a family line through the gradual development of an inferior stock, particularly as far as resistance to tuberculosis and cancer is concerned.

Levin, as well as Williams,<sup>3</sup> noted the family tendency to specific localization of the cancer, particularly of the uterus in the women, and of the gastro-intestinal tract in the men. This is well shown in my family histories and in some of the cancerous fraternities. Levin concludes that the most important result of his investigation is the fact that it shows the presence of an inherited resistance to cancer growth. I would put it in just the opposite way and say that my observations are important in that they show in certain families an inherited susceptibility to cancer. If the majority of the human race do not show this susceptibility, resistance to cancer is a normal trait of the species. An increased susceptibility becomes, therefore, the abnormal character of importance, and our investigations should be carried along the line of attempting to determine just what lies back of this susceptibility.

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3. Williams: *The Natural History of Cancer*, 1908, p. 364.



## A CLINICAL STUDY OF VAGOTONIA \*

ARTHUR H. HOPKINS, M.D.

PHILADELPHIA

In 1899 there appeared in the *New York Medical Journal* three admirable articles by Meltzer,<sup>1</sup> in which he deals with the function of inhibition. He speaks of the entire life of the animal as being a delicately adjusted equilibrium between excitation and inhibition and cites instances in which the slightest deviation of the resultant in the nervous mechanism of an organ may lead to the most serious consequences.

A later article<sup>2</sup> by the same author deals with the relation of inhibition to some forms of disease, and though it has paved the way for the more recent work brought forth by Eppinger and Hess<sup>3</sup> in Vienna, only the latter work will be discussed here.

It is my purpose to present a brief summary of their work together with a clinical analysis of a number of cases which I have studied during the last year. At the present time, though I am by no means prepared to support all of their hypotheses, the results are of interest and worthy of further study.

Owing to the difficulty of accurate diagnosis of neuroses, these authors have made a clinical study of such conditions, basing their work chiefly on varying conditions of irritability of the vagus and sympathetic nervous system. They divide the nervous system into the animal and vegetative, the former being represented by all the fibers running to voluntary muscles and sense organs, the latter by fibers supplying smooth muscle organs, as intestines, vessels, ducts of glands, etc.

Recognizing the difficulty in separating either anatomically or physiologically the fibers running to these organs, they attempted and succeeded in making a pharmacological separation, demonstrating the specific action possessed by epinephrin in stimulating the sympathetic system and the selective action of the pilocarpin group, i. e., pilocarpin, atropin, physostigmin and muscarin, for the vagus and vagus extended, or so-called autonomic system. The action of these drugs on the organs

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\* Read before the Section on Medicine of the College of Physicians of Philadelphia, February, 1913, and before the Germantown Branch of the Philadelphia County Medical Society, March, 1913.

\* Submitted for publication July 21, 1913.

\* From the William Pepper Laboratory of Clinical Medicine. Under the J. Alison Scott Research Fund.

1. Meltzer: *New York Med. Jour.*, May 13, 20, 27, 1899, lxi, 661, 669 and 739.

2. Meltzer: *Med. Rec.*, June 7, 1902, lxi, 881.

3. Eppinger and Hess: *Sammlung klinischer Abhandlungen über Pathologie und Therapie der Stoffwechsel und Ernährungsstörungen*, 1910.

involved is best demonstrated by Table 1. The accompanying diagram illustrates the autonomic and sympathetic systems anatomically.

Epinephrin being constantly secreted by the adrenals and general chromaffin system, must have a constant stimulating effect on the sympathetic system, and Eppinger and Hess<sup>3</sup> hold that it may be possible that a physiological analogue to epinephrin may exist for the autonomic nervous system. The central nervous system may be the general controller of the antagonistic systems and a disturbance of control, too much or too little irritability, too much or too little nerve tonus of one antagonist may bring about a pathological condition. On this ground they have attempted to clear up the so-called neuroses, studying clinically the irritability of the autonomic system, believing that a fluctuation in tonus or irritability might give an explanation for clinical symptoms.

TABLE 1.—ACTION OF DRUGS

Organs	Pilocarpin	Atropin	Epinephrin
Iris sphincter .....	Stimulates	Paralyzes	..... Stimulates
Dilator .....	.....	.....	.....
Ciliary muscle.....	Stimulates	Paralyzes	.....
Salivary glands .....	Stimulates	Paralyzes	Stimulates (?)
Sweat glands .....	Stimulates	Inhibits	Inhibits
Heart muscle .....	Inhibits	Stimulates	Stimulates
Vasomotor to head.....	.....	Contracts (?)	Contracts
Esophagus .....	Excites	Relaxes	Relaxes
Cardia .....	Excites	Paralyzes	Paralyzes
Stomach .....	.....	.....	.....
Tonus .....	Increases	Diminishes	.....
Peristalsis .....	Increases	Paralyzes	Paralyzes
Secretion .....	Increases	Diminishes	Diminishes (?)
Pancreatic secretion ....	Excites	Inhibits	Inhibits
Bronchi .....	Excites	Inhibits	Inhibits
Gall Bladder .....	Contracts	Relaxes	Relaxes
Intestinal Musculature...	Excites	Paralyzes	Paralyzes

High tonus in one system is accompanied by increased irritability in the other, and the antagonism must be also a pharmacodynamic one; individuals susceptible to epinephrin being only slightly susceptible to pilocarpin and vice versa. The name "*vagotoniker*" they apply to those constitutions which show functional increased tonus and increased susceptibility to pilocarpin, as well as an insusceptibility to sympathetic stimulation. The entire system or but a single branch may be involved. It finds its expression in latent increase of function and gives in this way to specific irritation a better point of attack than where no increase of tonus exists. Such specific irritation may arise from noxa in the form of bacterial toxins, as during or after acute infections, drugs or the products of deranged metabolism, mechanical irritation and so forth.

SYMPTOMS OF IRRITABLE VAGUS IN RELATION TO  
INDIVIDUAL ORGANS

*Eye.*—Ciliary muscle cramp, which is increased by pilocarpin and decreased by atropin; accommodation paralysis as observed after severe infection and finally strabismus may all be explained by vagal irritability.

*Salivary Glands.*—Salivation due to autonomic irritability may occur in nervous people, in *vagotonikers* and tabetic crises.

*Skin.*—Sweating is typical, and in crises of many infections, when associated with a slow pulse, suggests increase in tonus of heart vagus. Cold hands and feet suggest stimulation of dilators of peripheral vessels by the autonomic poison.

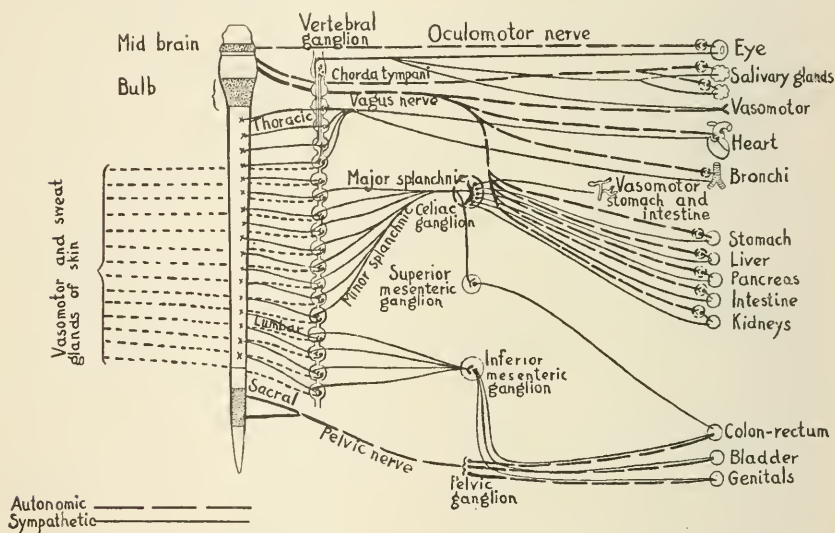


Diagram of the anatomical relations of the autonomic and sympathetic systems (Meyer and Gottlieb, *Experimentelle Pharmakologie*, 1911, p. 128.)

*Heart.*—Bradycardia is common in “vagotonikers,” especially in the young, and, owing to vagus irritation of toxic or mechanical origin, may be observed in convalescence after acute infections, icterus, brain pressure and many heart poisons.

Nervous block, as described by Hering,<sup>4</sup> is improved by atropin, while some forms of angina pectoris may be attributed to vagal irritability causing narrowing of coronary vessels.

After digitalis the early appearance of bradycardia with gastrointestinal disturbances is proof of special irritability in autonomic area. The combination of atropin with digitalis in such cases is held to be of great therapeutic value.

4. Quoted by Eppinger and Hess; see Note 3.

*Lung and Respiration.*—Bronchial asthma, which can be produced by peripheral irritation of vagus in animals, these authors assert is a classical example. Being due to a cramp of bronchial musculature, the air in the lung alveoli is not pressed out, the autonomic stimulation leads to muscle cramp with increase of secretion and further hindrance to air going out. Epinephrin checks secretion and atropin tends to relieve the spasm. Strumpell speaks of narrowing of the glottis in this condition, the recurrent laryngeal nerve being related to the autonomic system. Respiratory arrhythmia is common.

*Stomach.*—The vagus influences the form, peristalsis and secretion, and where there exists a physiologically high tonus there may be a great increase in gastric juice and hyperacidity without complaints. Pylorospasm with increased acidity, spasmodic hourglass constriction, cardiac and esophageal spasm, and finally vomiting accompanied by ptialism in the early stages of pregnancy may all be due to an irritable vagus.

*Intestine.*—Peristalsis is increased. There may be either diarrhea or constipation.

*Blood-Picture.*—Eosinophilia occurs especially where the skin, lung or intestinal element is present, and is increased after pilocarpin, often markedly.

*Urine.*—Rich in phosphates and oxalic acid, especially where there is high gastric acidity; may be slight dysuria.

The so-called vagotonic disposition is described by Eppinger and Hess as follows:

Often familial in type, this condition is seen most often in young people of nervous tendency who are subject to cold hands and feet, which are frequently bluish and mottled. They have a tendency to swallow when talking, to flush readily and to sweat readily; skin is moist, there is slight internal strabismus, increased power of accommodation, loss of sensation of touch in throat and larynx. Pulse is naturally slow. Marked respiratory fluctuations are present, due to irregularity in contraction of diaphragm. Reflexes increased, dermographism marked. The first complaint may be of gastric or intestinal disturbance.

#### COMBINATION OF VAGOTONIA WITH OTHER DISEASES

The presence of an irritable vagus, they say, may materially influence the symptoms in the following conditions:

Gastric ulcer or carcinoma, cholangitis, gall-stones, cholecystitis, stone in the kidney tract, tabetic crisis, hyperthyroidism, the menopause, puberty and menstruation, and skin conditions such as urticaria.

The advantage of atropin to combat anaphylaxis and the probable upset nervous mechanism in certain cases of tuberculosis due to destruction of epinephrin in chromaffin system are at least suggestive of a disturbance of this type.



## INTERNAL SECRETIONS

This question will not be dealt with beyond calling attention to the fact that epinephrin in the blood has an influence on the internal secretions of the pancreas, which in turn controls production of sugar in the liver. When there is too much epinephrin in the blood the internal secretion from the pancreas is inhibited and the liver can produce sugar excessively.

In cases of vagotonia there is a marked increase in tolerance for grape sugar up to 200 or 300 gm., while in cases of sympathetic neurosis there is a corresponding decrease in tolerance.

The test for vagotonia is as follows (Fleischmann):<sup>5</sup> Before the injection of pilocarpin the average pulse and respiratory rates are taken, the blood pressure estimated and smears made for a differential blood-count. Pilocarpin,  $\frac{1}{4}$  grain, is then given hypodermically and during the hour the general reaction is observed, as sweating, salivation, lacrimation, increase in nasal secretion, fibrillation, flushing, chills and cold extremities. The pulse and respiratory rates are taken every two or three minutes, the blood-pressure estimated at longer intervals and at the end of one hour more smears are made for a differential blood-count.

A strong general reaction accompanied by a marked increase in eosinophils in the blood, an increase in tolerance for grape-sugar up to 200 or 300 grams, and a cardiorespiratory arrhythmia may be interpreted as a positive result.

The following cases, having shown symptoms suggestive of an irritable autonomic system, were tested; Table 2 showing the results:

## REPORT OF CASES

CASE 1.—Diagnosis: cardiac extrasystole. Arrhythmia.

Capt. R. S., aged 45, presented himself five years ago complaining of attacks of palpitation of the heart or "flutterings" with missed heart-beats. Pulse in interval is slow and feet always cold. Patient is constipated when he stops using tobacco, which he has used to excess. During the attacks of fluttering which are brought on by slight exertion or mental excitement, he voids a large amount of urine.

Aside from the preceding symptoms he has no complaint. Physical examination and history are negative.

CASE 2.—Diagnosis: gastric neurosis.

K. G. F., female, aged 17, complains of regurgitation of food. Three years ago, just preceding onset of menstruation, patient developed regurgitation of food a few minutes after each meal, lasting for about five minutes. This continued for two years, when one year ago the attacks began to persist for about an hour at a time. Patient has a tendency to colds, nasal congestion, and is troubled with much nasal secretion; occasionally vomits freely. She has gaseous eructations toward end of regurgitations.

Examination shows tonsils large, hands and feet always cold and clammy. Roentgen ray shows curvature of spine to left from tenth dorsal to third lumbar

5. The technic of this test is the one used by Dr. P. Fleischmann in the Second Medical Clinic of Krankenhaus Charité, Berlin, to whom I am indebted for it.

TABLE 2.—TEST FOR VAGOTONIA IN TEN CASES\*

Before Pilocarpin					After Pilocarpin gr. $\frac{1}{4}$ †									
Case	Pulse	Resp.	Blood Pres.	Eos. Pct.	Pulse	Resp.	Blood Pres.	Eos. Pct.	Saliva c.c.	Sweat	Dext. Lev.	Flush	Fibril	Notes
1	22	4	142 58 115	0.5	27-21	3-4	155 70 120	2.5	++ 120 ++	Profuse	....	++	+	Chill
2	19	6	75 135	3.0	20-24	1-6	73 195	5.0	++ 260	Profuse	200 D 75 L	++	+	Nausea
3	16	4	75 122	53.0	23-18	2-5	80 133	65.5	++ ++	Profuse	200 D	++	+	.....
4	23	5	70 112	1.5	31-24	2-4	40 130	5.0	++ 124	Profuse	....	++	+	Nausea
5	22	5	90 154	3.0	25-20	4-7	... 150	7.0	++	Profuse	75 L	++	+	.....
6	25	6	120 112	0.0	29-24	4-7	85 130	14.5	++ ++	Profuse	75 L	++	+	.....
7	18	6	40	0.5	28-20	2-6	...	8.5	++ 100	Profuse	....	++	+	.....
8	16	5	... 115	....	21-17	3-5	... 115	3.0	++ 215	Profuse	200 D	++	+	.....
9	18	5	85 110	0.0	17-16	4-5	78 110	2.0	+	Moderate	....	+	—	.....
10	20	5	60	0.0	26-20	4-5	78	1.0	++	Moderate	....	+	—	.....

\* Cases 9 and 10 showing negative reactions are tabulated as controls.

† In all cases cold extremities followed the pilocarpin; no sugar.

vertebrae. After bismuth an increasing incoordination of peristalsis ending in faintness and vomiting. Fluoroscope showed on three examinations a fluttering at pylorus. Response to treatment good.

CASE 3.—Diagnosis: angioneurotic edema.

Mrs. B., aged 52, a brass-worker, since 1904 has been subject to localized edema every three or four weeks without fail. It is boggy, firm and does not pit on pressure; it gives a sense of pressure but no pain. In 1907 menstruation ceased. The attacks, which always occurred between the menstrual periods, have continued to date with almost the same regularity. The edema is localized to arms and face, and sometimes spreads to upper part of chest and lasts from four to eight days. Over thirty attacks have occurred in the University Hospital, sixteen being accompanied by convulsions. Urticarial eruptions frequently accompany the swellings.

Fifty-two blood-counts in 1908 showed an average eosinophilia of 60 per cent., while in 1909 there was an average of 59 per cent. They show an increase during an attack with reduction during the intervals.

CASE 4.—Diagnosis: angioneurotic edema.

J. L., schoolgirl, aged 14, last spring, two or three months before menstruation was established, first noticed localized swellings of upper eyelids and at times over brow. The swellings, which are boggy, do not pit on pressure and give no pain, have varied in size, being aggravated by overexertion or mental excitement, and decreased within twelve hours after patient has been in bed. She has had one urticarial wheal on foot. In the past few months she has noticed that her feet and hands are usually cold and feet often wet with sweat; there has been an increasing nasal secretion and marked constipation.

Mother had similar swellings which were especially bad during the menopause, at childbirth and at times when under severe mental anxiety; she has an enlarged thyroid and is of pronounced nervous temperament.

The patient on examination shows slight enlargement of right lobe and isthmus of thyroid, extremities cold, pulse somewhat rapid, waves irregular at times, knee-jerks increased, slight muffling of systolic sound at apex; otherwise no abnormalities detected.

CASE 5.—Diagnosis: fractured coccyx. Enlarged thyroid.

J. K., female, occupation, housework, complains of nervousness and enlarged thyroid. Patient has always had the swelling over the thyroid region unaccompanied by any symptoms until nine months ago, when she had a severe fall, landing on buttocks. Diagnosis of fractured coccyx was then made. Since the fall there have developed general nervousness and mental depression. For three weeks patient has daily periods of unconsciousness lasting from fifteen minutes to one half hour, preceded by chills. Cold and sweating of extremities warned her of attack each time. Patient never utters a cry and no convulsive movements have been noted in any seizure. Attacks are apparently vasomotor in origin. Three months ago patient had frequent attacks of vomiting. Patient is constipated.

Mother has similar swelling in neck.

Examination shows ocular symptoms negative, both tonsils enlarged, adenoids and symmetrical swelling of thyroid which moves with larynx in swallowing; reflexes very prompt; alimentary glycosuria is negative.

Transferred to surgical service, section of coccyx removed and patient discharged free from all symptoms except the thyroid enlargement.

CASE 6.—Diagnosis: Basedow's disease.

Mrs. S. O., aged 44, housewife, complains of swelling of neck, indigestion and palpitation of the heart. She presents the cardinal symptoms of hyperthyroidism, the onset of which followed an attack of rheumatism five years ago. She also complains of nausea, vomiting, eructations of gas and diarrhea.

Slight exophthalmos, lagging of upper lid and widening of palpebral angle are all present. Thyroid is enlarged and firm. Pulsation and palpable venous

thrill are present. Heart: soft systolic murmur at apex and both basal valve areas. Kidneys palpable. No alimentary glycosuria.

Section of thyroid at operation showed epithelial proliferation excessive.

CASE 7.—Diagnosis: bronchial asthma.

W. D., aged 28, drug clerk, eight years ago, following an attack of influenza, developed wheezing respiration, dyspnea and cough. He has had frequent recurrences since then, lasting from twenty-four hours to three months and typical of bronchial asthma. During attacks he is intensely nervous, voids urine frequently and has hot and cold flushes, and epigastric pain. He is greatly helped by epinephrin. Overeating and excitement precipitate attacks. Patient is constipated. He has had nasal catarrh with hypersection and sneezing for ten years; has had polyps and necrotic turbinate bone removed.

Patient given atropin and epinephrin to control attacks.

CASE 8.—Diagnosis: gastric neuroses.

R. C., aged 18, single, farmer, briefly presented the following condition: Gaseous eructations, pulse-rate 60, cold extremities, gastric hyperacidity, constipation.

Examination shows dermatographism, large tonsils, cold and mottled extremities, especially hands.

Of the few cases so far examined in which symptoms suggested the possibility of an increased tonus of the sympathetic system, but one gave a positive reaction.

This test, as carried out by Fleischmann, is as follows: The day before the test 100 gm. of dextrose are given on an empty stomach, and the first five hourly specimens of urine are collected, mixed and polarized. The following day another 100 gm. of dextrose are given, and one-half hour later 10 minims of epinephrin (1:1,000) are injected hypodermatically. Just previous to this injection, blood-smears are made for a differential leukocyte count and the average pulse and respiratory rate and blood-pressure estimated. After epinephrin the blood-pressure, pulse and respiratory rates are taken every two minutes, and the patient is observed for tremor and palpitation. At the end of one hour more smears are made for another differential leukocyte count. The urine is collected and polarized as after the first 100 gm. of dextrose. A positive reaction is obtained when there is a marked increase in lymphocytes at the end of one hour, a rise of at least 15 mg. mercury in blood-pressure, a decrease in sugar tolerance and the development of a tremor and sometimes cardiac palpitation.

The patient above mentioned came to the hospital complaining of bilateral paralysis of wrists, a tremulous voice and localized sweating over abdomen. He showed pigmentation of legs and peripheral olive-green staining of cornea, increased reflexes, some tremor and a reduction in sugar tolerance.

The results of his reaction are as follows: Blood-pressure increased from systolic 120 to systolic 140, lymphocytes from 28 per cent. to 43 per cent.; 9.1 gm. of sugar were recovered, as contrasted with a trace before injection of epinephrin. A markedly increased tremor of hands was noted, but no palpitation.



The presentation of the results of the cases, which includes less than half of the series studied, is made with the view of approaching a little nearer to an accurate diagnosis of those diseases so frequently filed away in the records of even the best-regulated hospitals, as neurasthenia, gastric neurosis, etc.

It must be admitted that hypotheses are abundant, and as has been said, I have not been able to support all of them by the analyses of these few cases; certain of them are, however, confirmed, and this newer method of approaching a clear-cut diagnosis is not only of interest, but is worthy, I believe, of still further investigation and development.

Since starting this work my attention has been attracted to the study of a series of cases presented by Barker<sup>6</sup> in 1912, to an article by Neuhof<sup>7</sup> in the same year in which he discusses reflex vagus phenomena, but does not deal with the question of drug reactions, and to Abrams'<sup>8</sup> text-book on spondylotherapy.

In conclusion I wish to extend my thanks to Dr. Stengel for the opportunity of studying the cases, nearly all of which were on his service in the University Hospital, and to Dr. H. B. Wilmer for case 4.

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6. Barker and Sladen: *Tr. Assn. Amer. Phys.*, 1912, xxvii, 471.

7. Neuhof: *Am. Jour. Med. Sc.*, May, 1912, cxliii, 724.

8. Abrams: *Spondylotherapy*, 1912.

# EXPERIENCES WITH PROPHYLACTIC TYPHOID VACCINATION

ITS EFFECT ON MENSTRUATION \*

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NEW YORK

Prophylactic typhoid vaccination has won for itself a well-deserved place among the great preventive measures in medicine. This advance has been accompanied by a considerable literature on the history of the procedure, the various methods employed and the results obtained. It is not the object of this article to review the literature. The facts are easily accessible,<sup>1</sup> and, in general, well known.

During the past two years the vaccine has been used prophylactically at the Presbyterian Hospital, New York, and it is the purpose of the present communication in reporting the results of this experience, to emphasize the facts which have seemed most important and to point out in some detail the especial effect of the vaccine on menstruation.

## PREVIOUS CONDITIONS

Stimulated by the excellent results obtained by this procedure in the United States Army and by Richardson and Spooner in the training schools of Massachusetts, the medical board of the Presbyterian Hospital, New York, decided, in the spring of 1911, to offer this opportunity of immunization against typhoid to the nurses and attendants at the hospital. Before starting the inoculations, the hospital records were reviewed in order to ascertain the incidence of the disease among those brought into intimate contact with typhoid patients or the cultures of typhoid bacilli. Beginning with the year 1892, the date of the foundation of the training school for nurses, a period of twenty years was covered, during which

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\* Submitted for publication, Aug. 2, 1913.

\* From the Pathological Laboratory, Presbyterian Hospital, Columbia University, New York.

1. The following articles give the main facts of interest and contain references to most of the other important papers:

Russell, F. F.: The Military Surgeon, 1909; Boston Med. and Surg. Jour., 1911, clxiv, 1; Jour. Am. Med. Assn., 1912, lviii, 1331; 1912, lix, 1362; 1913, lx, 344; 1913, lxi, 666; Harvey Lecture, New York, 1913.

Spooner, L. H.: Boston Med. and Surg. Jour., 1910, clxii, 37; Jour. Am. Med. Assn., 1912, lix, 1359.

Davis, D. J.: Jour. Am. Med. Assn., 1912, lviii, 537.

Hachtel and Stoner: Jour. Am. Med. Assn., 1912, lix, 1364.

fifty-two cases of typhoid fever in nurses, doctors, orderlies and bacteriologists were treated in the hospital. Only those cases were included which gave a direct history of contact with typhoid patients or cultures. This gave 2.6 as an average yearly percentage of cases in which typhoid was possibly preventable. There were four deaths, a mortality of 7.69 per cent.

The cases were divided as follows: nurses, 39; orderlies, 6; bacteriologists, 4; doctors, 3.

Of the thirty-nine nurses, nineteen, or about one-half, were in training or on duty in the hospital at the time of contracting the disease. The remaining twenty were caring for typhoid patients when taken ill. There were no deaths among the nurses who developed the disease while in the hospital, but of those who contracted the disease outside, two died. Both were graduates of the hospital. All six orderlies were employed at the hospital in wards in which the care of typhoid patients was necessitated, and there were two deaths. The four bacteriologists developed the disease while working with typhoid cultures. Only one was on duty at the hospital at the time of contracting the disease. The others came from other laboratories. There were no deaths. Of the doctors, two were interns at the hospital and one was a substitute. There were no deaths.

The occurrence of the fifty-two cases by years was as follows:

1892 .....	1	1902 .....	1
1893 .....	0	1903 .....	3
1894 .....	0	1904 .....	4
1895 .....	1	1905 .....	5
1896 .....	2	1906 .....	3
1897 .....	0	1907 .....	6
1898* .....	11	1908 .....	1
1899 .....	1	1909 .....	0
1900 .....	4	1910 .....	3
1901 .....	4	1911 .....	2

\* Spanish War.

It will be seen that in four separate years, 1893, 1894, 1897 and 1909, there were no cases, and that for one period of two years there was also a clean record, facts which must be borne in mind in estimating the value of the results obtained through the use of the vaccine over a comparatively short period of time. In 1898, the year of the Spanish War, there were eleven cases. At that time additional wards were opened for the care of typhoid patients and the hospital staff was greatly overworked. Several of the nurses treated at this time came from the army camps.

The conditions described do not differ essentially from those reported by observers in other localities. Thus Spooner,<sup>2</sup> at the Massachusetts General Hospital in Boston, found that there were three or four cases of

2. Spooner: *Am. Jour. Pub. Hyg.*, 1909, xix, 616.

typhoid a year among the nurses and those especially exposed. He found, moreover, that in these patients the disease ran a course of more than usual severity with a greater number of complications and a higher mortality than the average. I did not find this true in our statistics. The mortality was less than that for the hospital in general and the cases were not unusually severe, nor did they show more complications than the average.

Davis<sup>3</sup> gives no definite figures for St. Luke's Hospital, Chicago, but states that "an occasional nurse has had typhoid."

Joslin and Overlander<sup>4</sup> investigated the conditions in six hospitals in Massachusetts for the years 1902 to 1906 and came to the conclusion that one nurse came down with typhoid for each 114 cases treated, and that "the hospital nurse in Massachusetts is about eight times as liable to contract typhoid fever as the ordinary citizen."

Hachtel and Stoner<sup>5</sup> give higher figures for Baltimore. They find that the nurses and attendants are from twelve to twenty times more liable to contract the disease than the ordinary citizen of Baltimore.

One does not need to cite further specific instances. While differing somewhat in the actual figures, all observers are agreed on the main fact, that nurses, doctors and hospital attendants run a considerably greater risk of contracting typhoid than the ordinary citizen, and that in spite of due precautions, a certain number of them contract the disease each year, the source of infection being the typhoid patient or articles which have come in contact with typhoid cases.

#### VACCINE

The vaccine used is prepared from an old culture of attenuated virulence, isolated about twelve years ago. The organisms are grown on large agar-slants (agar slanted in 250 c.c. Erlenmeyer flasks is very convenient) for twenty-four hours, washed off with sterile salt solution and heated at a temperature of 53 C. (127.4 F.) for one hour. The vaccine is standardized with the ordinary blood-counting apparatus. After sterility is assured, the vaccine is made up to the required dilution with sterile salt solution containing 0.25 per cent. phenol (carbolic acid). It is put up in small ampules, each containing enough for one dose, and in 30 c.c. vaccine bottles for use where larger numbers are to be vaccinated.

The vaccine was carefully compared with that used in the United States Army, a supply of which was very kindly sent to the hospital by Major Russell. In a large number of inoculations no difference could

3. Davis: Jour. Am. Med. Assn., 1912, lviii, 537.

4. Joslin and Overlander: Boston Med. and Surg. Jour., 1907, clvii, 247.

5. Hachtel and Stoner: Jour. Am. Med. Assn., 1912, lix, 1367.



be detected in the local or general reactions or in the Widal reactions. Therefore, it would appear that the hospital vaccine represents approximately the same potency and lack of toxicity as that used in the army. The vaccine should be fresh and in general should not be used after it is 3 months old, as it has been shown that deterioration begins at about this time.

#### DOSAGE

The usual dosage recommended is that employed in the United States Army. It consists of three inoculations at ten-day intervals, the first of 500 million, the second and third of one billion each. This method has been well tried and it has been conclusively shown that it confers a sufficient immunity for a period of at least three years. Conditions there, however, differ somewhat from those among nurses and in private practice where a reaction which would not trouble a man in the regular army is considered quite an event. The same dosage as previously noted was given in a considerable number of the cases in this series, and there were enough troublesome reactions to make one consider a variation of the dose. Of course, one's aim in giving the vaccine is to protect the patient against typhoid and not to avoid reactions; but, if one can produce the desired immunity with the smallest amount of discomfort, the cause of typhoid prophylaxis has been aided just so much. The aim, then, should be to inject the maximum number of bacilli with the minimum amount of discomfort and inconvenience to the patient.

One class of nurses received the four doses, recommended by Spooner,<sup>6</sup> of 100 million, 200 million, 400 million and 600 million at five-day intervals. These cases had fewer and less troublesome reactions than the others, but they received only about one-half the total number of bacilli given by the army method. By considering certain general factors to be mentioned presently, one can considerably increase this total without a corresponding increase in the reactions. It is generally quite safe to make the initial dose 200 million and then, being governed by the reactions which occur, one can very often make the second dose 300 to 400 million, the third 500 to 600 million and the fourth 800 million to one billion. In this way one can more nearly approximate the army total dosage without as many disagreeable reactions.

Some people prefer to take their chances on the possibility of having a reaction rather than submit to the inconvenience of a fourth injection, and in vaccinating large numbers, the extra inoculation means considerable added expenditure of time, especially among women in whom the avoidance of the menstrual period causes many delays and much straggling out of the inoculations. Therefore, it has been the more recent routine to use three graduated doses at from seven-day to ten-day

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6. Spooner: *Jour. Am. Med. Assn.*, 1912, lix, 1359.

intervals, the first of 300 million, the second of 700 million and the third of one billion. Here, as in the preceding case, one can be governed by the patient's general condition and can often without fear of undue disturbance, make the first dose 350 to 400 million and the second 800 million.

Of course, it is impossible to predict accurately what reaction a person will have, but one can be governed by certain general facts. In the first place, women are more apt to have reactions than men. This fact is brought out in the statistics of Hachtel and Stoner,<sup>7</sup> and was shown in the first twenty-three cases of this series, of whom twelve were doctors and eleven were nurses. The mild and moderate reactions were about twice as frequent among the latter as among the former. Members of families in which there is, apparently, a marked susceptibility to typhoid or in which the disease has run a severe course, are apt to show more marked reactions. In one family which I vaccinated, both of these facts were true, and of the five members, all showed varying degrees of reaction after all of the inoculations except a boy, aged 11. In one there was the most severe local reaction which I have seen, combined with a moderately severe general reaction and in another, the general reaction after the first two doses was sufficient to discourage a third inoculation. Persons who have a low resistance to any form of infection are apt to have troublesome reactions and the same is true of those who are run down or debilitated from any cause. Those suffering from any chronic disease, should, if vaccinated at all, receive the four smaller doses. Naturally, the preceding statements are only general principles of guidance. One will encounter perfectly robust individuals who come in none of the foregoing classes, who, nevertheless, show severe reactions, while, on the other hand, any of the types mentioned may show no reaction whatever. From my experience up to the present time, I believe that it is best to give to the persons of the types mentioned, the four doses at seven-day intervals, beginning with 200 million and increasing each succeeding dose as far as feasible. For all others, I prefer the three graduated doses, using the upper limits for each dose if possible. From a rather limited experience with children I have seen no reason for graduating the dosage according to body weight. As a rule, they stand the inoculations with less inconvenience than adults and I have seen no troublesome reactions following full-sized doses.

With many people, especially business men, a seven-day interval is preferable to one of ten days. The inoculations can thus be given on successive Saturdays, allowing them until Monday to recuperate from any ill effects. It is best where possible to give the injections in the

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7. Hachtel and Stoner: *Jour. Am. Med. Assn.*, 1912, lix, 1367.

late afternoon or early evening so that the maximum reaction may take place while the patients are asleep.

#### PRECAUTIONS

From my experience it has been apparent that whatever the site chosen for inoculation the patient is inclined to wish that the vaccine had been injected elsewhere. It has been found more convenient and perfectly satisfactory to make the inoculation in the left upper arm, just above the insertion of the deltoid, using the right arm in left-handed people. The vaccine is injected into the subcutaneous tissue, care being taken to avoid entering a vein, as in such cases the general reaction is usually more rapid in its appearance and more severe, and the local reaction more troublesome. In cleaning the arm, iodine and alcohol have been found perfectly satisfactory. After withdrawing the needle, the site of inoculation is touched with iodine and no dressing of any kind is applied, as it has been found that collodion, bandages or gauze with adhesive strips simply add to the local annoyance. In exceptional cases, if the local reaction is moderately severe, a dressing of aluminum acetate has been used with good results.

In prophylactic work, one must remember that patients are not inclined to be particularly grateful for any reactions which the vaccine may cause and it is only human nature for them to attribute to the vaccine every ill for months following. As the value of typhoid vaccine is so great and the need for its universal adoption so striking, it is of the utmost importance to observe every precaution to prevent its falling into disrepute in any case. A careful history should be taken in each case and a sufficiently exact examination made to be sure that the patient is in sound health, for it is quite within the range of possibilities that a person might be inoculated in the early stages of some disease, and it would then be difficult to convince the patient or his friends that the disease was not directly caused by the vaccine. In the present work such incidents were encountered. One nurse developed a typical case of scarlet fever two days after the second inoculation, and for the first day or two even the doctor who saw her considered that she was suffering from a rather severe vaccine reaction with an associated rash. From the subsequent course of the case, there was no doubt as to the nature of the disease. In such an instance in private practice, it would be exceedingly difficult to convince the family that there was no association between the vaccine and the scarlet fever. In another case, a man had an attack of bronchopneumonia which began ten days after the third injection. At the onset he believed that his illness was in some way associated with the vaccine, though in this case the disease followed a very definite exposure to cold. Other cases of association of various diseases with the vaccine will be given later.

It is well recognized that the vaccine may cause a temporary change for the worse in an existing chronic condition. For example, Spooner<sup>8</sup> has encountered temporary and not serious exacerbations in chronic arthritis, chronic cholecystitis, subacute urethritis, furunculosis and acne. I have seen similar instances and will mention them later. Other observers have noted the same thing. While the exacerbations are not apt to be serious, their possibility should be recognized and all chronic conditions should be asked for in the history, as in each case one can then decide whether or not it is wise to give the vaccine. I do not believe that it should be given in cases in which there is a history of tuberculosis; but if, in spite of the existence of some chronic condition, it is decided to go ahead with the vaccine, the patient should be fully acquainted with the possibilities and the vaccination procedure should be followed cautiously and the four smaller doses administered.

Another question which arises is that of vaccination where there is a history of a preceding attack of typhoid fever. It has been found that the reactions in such cases are apt to be rather strikingly severe. In the only case of the kind in which I have used vaccination, that of a young man, there was a moderately severe reaction for a day and a half after the first dose, followed immediately by a severe attack of tonsillitis and in ten days by a profuse urticarial rash. As the disease itself confers a life immunity in almost all cases, and as vaccination in such cases is apt to be followed by bad reactions, I have since refused to inoculate patients who give a history of a previous attack of typhoid. At times this preceding history is not definite and in such cases one must exercise one's own judgment. Where there is a reasonable doubt, I believe one should proceed with the vaccination, using the four smaller doses rather cautiously.

In addition to the above precautions, one should explain the effects of the vaccination to patients and tell them just what they may expect in the way of local and general reactions. Patients should be warned to remain quietly at home while the reaction is present. The most severe reaction seen in the present cases seemed to have been increased by the patient's going about as usual on the day following the first inoculation. when the temperature was 101. The next day the temperature rose to 102.6, and in connection there was a splitting headache and slight epistaxis, while on the following day the temperature reached 100, with the accompaniment of nausea and rather severe diarrhea. Patients should also be warned against taking alcohol in any form until all signs of reaction have disappeared, for not infrequently alcohol has apparently added considerably to the severity of the reaction. In this connection, a curious fact was noted in three of these cases. Two patients on the

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8. Spooner: *Jour. Am. Med. Assn.*, 1912, lix, 1360.



night following inoculation and one on the second night after, when there was no general reaction and their arms had ceased troubling them, took cocktails just before dinner. Almost immediately afterward their arms, at the site of the inoculation, began to pain so severely that they could not remain at the table.

#### RESULTS

The inoculations were started in May, 1911. The course of procedure was carefully explained and eleven nurses and twelve doctors volunteered for the preliminary vaccinations. In these twenty-three cases, only two inoculations were given, the first of 400 million and the second, after an interval of ten days, of 650 million. In seventeen of these cases, microscopic Widal's were done ten days after the first injections, the Widal's prior to the vaccination having been negative in all cases at 1:20. In five the Widal was negative in a dilution of 1:20, in twelve it was positive and in two it was positive at 1:100. The results of the Widal test following the second inoculation may be seen in Table 1.

TABLE 1.—RESULTS OF WIDAL TEST IN TWENTY-TWO CASES\*

Dilution	Reaction	Number	Percentage
1-20	Negative	3	13.63
1-20	Positive	19	86.36
1-100	Positive	12	54.54
1-200	Positive	6	27.27
1-400	Positive	6	27.27
1-1600	Positive	1	4.54

\* Tests made ten to fourteen days after second inoculation.

After these preliminary inoculations, the work was taken up as a routine with each succeeding class in the training-school (Table 2). During the first few months the members of a new class are not on ward duty, and as some leave the school and others enter, the vaccinations are not started until the nurses have been here for several months. Then the class is called together, the vaccination and its importance explained and the vaccine offered to those who desire it. Vaccination is still entirely voluntary in the training-school.

The following table shows the percentage of each class vaccinated:

TABLE 2.—PERCENTAGE OF VACCINATIONS OF NURSES

Class of	In class	Number eligible	Vaccinated	Percentage
1911.....	29	26	16	61.5
1912.....	33	31	22	70.9
1913.....	38	32	27	84.3
1914.....	13	11	11	100.
1915.....	30	25	23	92.
Totals.....	143	125	99	79.2

This table shows that as the work became better known and appreciated, a larger number of the nurses wished the vaccination. Thus in the last two classes, 94.4 per cent. of those eligible for the vaccination have taken it.

Since May, 1911, 143 nurses have been in training. Of these, 99 were vaccinated, 26 refused; in 3 it was considered inadvisable on account of some form of chronic illness, 14 had recovered from typhoid and one had been inoculated with the vaccine before entering the school. Thus, 79.2 per cent. of the eligible nurses were vaccinated and 20.8 per cent. remained unprotected, including the three who wished the vaccine, but whose health did not warrant its use.

In addition, during the same period, May, 1911, to June, 1913, 33 doctors and medical students connected with the hospital, 7 employees in the pathologic department, where the vaccine is compulsory, and 64 outside cases have been vaccinated, making a total of 203 cases; and in this period there have been no cases of typhoid fever either in the training-school or among those vaccinated. There have been no untoward effects from the vaccine and no instances of arm infection.

#### LOCAL AND GENERAL REACTIONS

*Local and General.*—The local reactions were similar in all respects to those usually observed.

The general reactions are shown in Table 3. In classifying these reactions, the temperature is taken as the general index as in army cases, but some reactions are put down as mild or moderate where the constitutional disturbances were sufficient to warrant it even though the temperature remained low. It was noted that some of the nurses showed subnormal temperatures following the inoculations, which was often associated with such constitutional disturbances as dizziness, nausea and vomiting.

TABLE 3.—PERCENTAGE OF GENERAL REACTIONS

Reaction	First Dose 203 Cases		Second Dose 200 Cases		Third Dose 169 Cases		Fourth Dose 27 Cases	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
None.....	96	47.6	111	55.5	140	82.8	24	88.8
Mild.....	88	43.3	71	35.5	19	11.2	3	11.1
Moderate..	18	8.9	18	9	11	6.5	0	....
Severe.....	1	0.49	0	....	0	....	0	....

It will be noted that of these cases, 184, or 90.6 per cent., showed either no reaction or a mild one after the first dose; 182, or 91 per cent., after the second; 159, or 94.1 per cent., after the third, and 27, or 100 per cent., after the fourth. The symptoms complained of in the order of frequency were: malaise, headache, general aching, nausea, dizziness, anorexia, vomiting, diarrhea, epistaxis, faintness.

One hundred and ninety reported at the end of six months that they were perfectly well, three had suffered from fatigue for two or three

months, but had recovered, and two thought that their health was still impaired by the vaccine.

#### WIDAL REACTIONS

The results of the microscopic Widal in fifteen consecutive cases tested ten days after third inoculation, may be seen in Table 4.

TABLE 4.—RESULTS OF WIDAL TEST IN FIFTEEN CASES\*

Dilution	Positive Reactions	
	No.	Per cent.
1-20	15	100
1-100	15	100
1-200	11	73.3
1-400	9	60
1-800	5	33.3
1-1600	3	20
1-2000	2	13.3
1-12000	1	6.6

\* Test made ten days after third inoculation.

The Widal reaction has been so definitely worked out by so many observers that it is now used here only in exceptional cases and to control new vaccine.

#### MENSTRUATION

After the first few inoculations the very definite impression was gained that menstruation was apt to be affected by the vaccine. Consequently, careful records of this particular phase of the work were kept in order to determine the amount of the disturbance. A comparison of the army vaccine with that used in the hospital showed no difference in their liability to cause menstrual irregularities. Other workers have briefly noted that these irregularities occur, so that one is justified in concluding that the effects are not due to the peculiarities of any one particular vaccine. A correct judgment of the part played by typhoid vaccine in menstrual disturbances is difficult and any statistics are open to several sources of error.

In the first place, it is well known that nurses are apt to have menstrual irregularities during the first few months of training, which are due to change of environment and method of living. In this work, the vaccinations were not started until the nurses had been in the hospital for from three to six months, and these nurses did not show any more frequent or more marked changes than did the nurses who had been in training for from one to three years. Again, some women are always more or less irregular. Where there was such a history, any disturbances during the course of the inoculations, unless of striking character, were not considered, and the case was put down as unaffected. In some, temporary causes other than the vaccine may have been the causal factors. It is difficult to eliminate such causes and they introduce a certain unavoidable source of error.

In compiling the records of the one hundred cases in which menstruation was studied, only those cases are put down as affected in which the evidence of disturbance by the vaccine seemed to me quite definite, after ruling out all other apparent causes and where the patients themselves felt that the condition was distinctly unusual for them.

Perhaps one can gather the best idea of the nature of the disturbances and their close association with the vaccine by a brief description of some of the more striking cases.

#### REPORT OF CASES

CASE 1.—Vaccination finished early in June. The two following periods were missed. In August appendix was removed and menstruation did not occur again until December. It has been normal since then.

CASE 2.—For three periods following the vaccination was about three weeks late each time. Then normal.

CASE 3.—Missed period following vaccination and then a condition of scanty flow a week or more late existed for three months.

CASE 4.—Menstruated every two to three weeks for three months following the vaccination.

CASE 5.—After the third inoculation came on two weeks ahead of time and caused more discomfort than ever before.

CASE 6.—Vaccinated the day after menses had ceased. Menses returned again that night.

CASE 7.—During the course of vaccination menses came on one week ahead of time, causing patient to go to bed.

CASE 8.—Twelve days early. More profuse and of longer duration.

CASE 9.—After first inoculation menses ten days early, more painful and scantier.

CASE 10.—After third inoculation menses ten days early, more painful and scantier.

CASE 11.—Never quite regular. Much worse after vaccination. Skipped two months entirely and later menstruation was more painful.

CASE 12.—Five days late after the second inoculation. One week early after the third.

CASE 13.—Skipped two periods. Next time in three weeks. Then in less than three weeks.

CASE 14.—Skipped three periods.

Thus in fourteen cases there were very distinct changes, due, in all probability, to the effect of the vaccine itself.

A complete tabulation of all the cases shows that a little more than one-half, 53 per cent., showed some type of menstrual disturbance, distinct though at times quite trivial, while 47 per cent. were unaffected. The types of disturbance may be seen in the following table, more than one type occasionally appearing in the same individual:

Menstruation early for one or more periods.....	25
Menstruation more painful for one or more periods.....	18
Menstruation late for one or more periods .....	15
Menstruation more profuse for one or more periods.....	10
Menstruation skipped for one or more periods.....	7
Menstruation scantier for one or more periods.....	6



It should also be noted that where these irregularities occur the patients are more apt to have associated with them a more troublesome general reaction.

The number of cases which show some change is rather striking, even allowing for unavoidable errors. If one merely considers the fourteen very definite instances cited, the percentage is high enough to show that a very striking relationship exists between the vaccine and menstrual disturbances, a fact which is of interest in view of the similar disturbances seen in typhoid fever. All of the cases included above were followed for at least six months. In none was there any disturbance at the end of this time. Therefore, it would appear that the changes are of only temporary significance and that they carry with them no lasting ill effects.

In this connection, one must consider the possible consequences of vaccination during pregnancy. I have a record of only one such case. In this instance, the woman's husband was ill with typhoid and much of his care fell on her. Consequently, in spite of her pregnancy, vaccination was carried out. She did not develop typhoid, nor did she have any ill effects from the vaccine. The menstrual disturbances have impressed me sufficiently, however, to make me conservative about vaccinating pregnant women. At the present time, I would not vaccinate under such circumstances, except under some special indication, such as that shown in the preceding case.

Recognizing that menstrual disturbances do occur, one naturally turns to the question of reducing them to a minimum. I believe that, barring some special indication for haste, the vaccination of women should be so arranged that the first inoculation will come a few days after a period. The second and third injections can then be given at seven-day intervals, leaving about a week's leeway before the succeeding period. If, for any reason, ten-day intervals are necessary, it is best to postpone the third inoculation until a few days after the second period. By giving the vaccine in this way, menstrual difficulties are reduced, though not entirely eliminated.

#### ASSOCIATION WITH OTHER DISEASES

It has already been mentioned that numerous instances of aggravation of existing chronic conditions are on record. It is, therefore, of interest in a series of cases like the present, where most of the cases can be followed, to note the relation of the vaccine to the development of various diseases.

*Diseases of the Respiratory Tract.*—Thirteen patients developed symptoms of some such affection during the course of the vaccinations, or so soon afterwards that they attributed them to the vaccine. Five patients developed coryza beginning within one or two days after an

injection, and in one case the cold lasted for six weeks. There were five cases in which tonsillitis began within one or two days after an inoculation. In one case bronchitis developed during the course of the vaccination, and the disease lasted for two months. One patient, already referred to, contracted bronchopneumonia ten days after the third injection. In this case there was definite exposure to cold.

Probably many of these cases were merely coincidences, though one receives the very definite impression that in some cases, at least, the patient's resistance is sufficiently lowered to favor the chances of such infections.

*Appendicitis.*—This condition was noted in five cases. In only one, however, was the association sufficiently close to justify suspicion of the vaccine as an accessory cause. In this case, there were moderately severe reactions associated with nausea, vomiting and diarrhea, after all three doses. Very soon after the third dose the patient began to have pain in the region of the appendix, which in the course of a short time necessitated an operation.

*Tuberculosis.*—Three patients in the present series have developed pulmonary tuberculosis since receiving the vaccine. In no case was there any previous history of the disease, and in none was the relation between the vaccine and the disease sufficiently close to be suggestive.

*Tachycardia.*—In one case there was a history of mild attacks of tachycardia. The patient had two such attacks on the eleventh and twelfth days following the second inoculation, and a third attack within five hours after the third injection. In another case, that of a man who normally had a very slow pulse, the rate doubled within a few hours after receiving the first dose. In a third case with a history of attacks of rapid heart action and arrhythmia, there was no effect from the vaccine.

*Furuncles.*—In one instance a patient began to suffer from furuncles about two weeks after the third injection.

*Scarlet Fever.*—The development of a case of scarlet fever during the course of the injections has already been mentioned.

*Scabies.*—One patient was suffering from a rather extensive case of scabies, when he presented himself for the vaccination. He was warned that the vaccine might cause an aggravation of the skin condition, but he desired the vaccine in spite of this possibility. Much to our surprise, without any treatment for the skin affection, it almost completely disappeared during the course of the inoculations, but reappeared a little later.

# HEXAMETHYLENAMIN: THE LIBERATION OF FORMAL- DEHYD AND THE ANTISEPTIC EFFICIENCY UNDER DIFFERENT CHEMICAL AND BIOLOGICAL CONDITIONS \*

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Ever since the recognition of hexamethylenamin as a beneficial therapeutic agent in vesical conditions by Nicolaier<sup>1</sup> in 1894, the use of this drug has steadily increased. It has been recommended as an antiseptic agent for practically all of the body fluids, largely on the basis of the fact that after internal administration it has been found present everywhere in the body. Although it has been quite generally recognized that hexamethylenamin is practically non-toxic, the criteria for its action as an antiseptic have only comparatively recently begun to be appreciated. Its decomposition into ammonia and formaldehyd is well known, and it is generally believed by pharmacologists that the bactericidal properties of hexamethylenamin are due to the liberated formaldehyd. However, there has been no absolute proof that free hexamethylenamin may not prevent bacterial growth. Indeed, it is maintained by some clinicians that hexamethylenamin is bactericidal and that the presence of free formaldehyd is not necessary when it is to be used as an antiseptic.

The conditions under which formaldehyd may be liberated from hexamethylenamin have been ascertained largely from experiments *in vitro*. Some of the factors are changes in temperature; time allowed for the decomposition to take place, and reaction of the solution. The last is, undoubtedly, the most important factor. In general, acids are known to facilitate its decomposition, while alkalis prevent it. There has been no thorough study of the influence of these factors under conditions simulating those of the body, and especially in the living organism. This is especially necessary in the light of the modern conceptions of the true reactions of the various body fluids, according to Sørensen, L. J. Henderson and others. Tests for the appearance of hexamethylenamin and formaldehyd in urine and other body fluids have been quite

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\* From the Pharmacological Laboratory, Medical School of Western Reserve University.

\* Submitted for publication August 15, 1913.

\* The expenses of this research were defrayed in part by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

1. Nicolaier, A.: Deutsch. med. Wehnschr., 1895, No. 34, p. 541; Centralbl. f. d. med. Wissensch., 1894, xxxii, 897.

extensively employed, but often with confusion as to their delicacy and clinical applicability, and without due appreciation of their worth and significance. Tests have been accepted as conclusive for formaldehyd which do not differentiate between free hexamethylenamin and free formaldehyd; certain tests are thought to be best suited for clinical application, others for pure chemical work only. Therefore, we have attempted to gain more definite information concerning the applicability of various tests for free formaldehyd and free hexamethylenamin; to study the various conditions under which the liberation of formaldehyd takes place, and to settle definitely whether hexamethylenamin itself is bactericidal or not.

In this work we have sought to correlate as much as possible the chemical data obtained with experiments *in vitro* and on the lower animals with observations on patients. The clinical material was obtained from the medical wards and dispensary clinics of Lakeside Hospital and Western Reserve University. For a description of the methods used, the sections to follow must be consulted.

The more important results of our work may be briefly stated as follows: The phloroglucin test is the most delicate and most useful test for free formaldehyd. Free alkali prevents the liberation of formaldehyd from hexamethylenamin. Liberation of formaldehyd from hexamethylenamin depends on the hydrogen ion concentration of the solution; that is, it depends on true acidity. After the administration of hexamethylenamin, free formaldehyd does not occur in blood, cerebrospinal fluid and all other body fluids which are truly alkaline (concentrations of hydrogen ions less than 7.0), nor does it occur in alkaline urine; but it always occurs in the urine of animals and of man when the reaction is acid, that is, when the hydrogen ion concentration is greater than 7.0 (neutrality). Definite and direct proof is offered that hexamethylenamin itself is not bactericidal.

### *I. Tests for Formaldehyd and Hexamethylenamin*

*Bromin-Water Test for Free Hexamethylenamin.*—This test is described by Nicolaier<sup>2</sup> as follows: Fresh bromin-water gives an orange-yellow precipitate when added directly to solutions containing hexamethylenamin. The precipitate forms at the moment of contact with bromin water, but redissolves until three to four drops of the reagent have been added. The precipitate forms when the merest trace of hexamethylenamin is present. No precipitate is formed with free formaldehyd. The test is applicable to urine, but to no other body fluids containing proteins such as blood, blood-serum, cerebrospinal fluid, pleural, pericardial and synovial fluids and urine containing albumin, because the proteins themselves form a precipitate. In order to test for hexamethylenamin in fluids in which the bromin-water test is not applicable, the fluid is distilled alone or with the addition of a little mineral acid and either of the following tests for formaldehyd are applied to the distillate.

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2. Nicolaier: *Ztschr. f. klin. Med.*, 1899, xxxviii, 350.



*Tests for Free Formaldehyd.*—Many of the most widely employed tests for free formaldehyd involve the use of acids, which would decompose the hexamethylenamin, and would therefore be unsuited for distinguishing between free formaldehyd and hexamethylenamin. Only those which are made in weakly alkaline reaction are suitable. This limits the choice practically to the phenylhydrazin and phloroglucin tests.

*Phenylhydrazin-Nitroprussid Test for Free Formaldehyd.*—This test was first described by Rimini,<sup>3</sup> and later by Arnold and Mentzel.<sup>4</sup> Three reagents are necessary: (1) Phenylhydrazin hydrochlorid 0.5 per cent. in water; (2) sodium nitroprussid<sup>5</sup> 5 per cent. in water; (3) sodium hydroxid 10 to 20 per cent. The test is performed in the cold or at ordinary room temperature as follows: To 5 to 10 c.c. of the fluid to be tested contained in a test-tube are added 3 drops of the phenylhydrazin, 2 drops of the nitroprussid and 3 drops of the alkali in the order mentioned. If formaldehyd is present in an aqueous solution, an emerald green to a deep blue color is formed (depending on the concentration of formaldehyd) at the moment the alkali comes in contact with the fluid. This color gradually diffuses throughout the fluid and almost at once begins to disappear, particularly in highly dilute formaldehyd solutions, assuming finally an orange-yellow to a wine-red color. With water alone, the test gives a greenish-yellow at the moment the alkali is added, making it entirely indistinguishable from very dilute solutions of formaldehyd (1:2,000,000 and higher). The change to wine-red occurs much more rapidly in water than in solutions containing formaldehyd which are positive with the test. In urine containing formaldehyd the sequence of colors is as follows: As soon as the hydroxid is added, a deep purple color (generally, but not always) is formed. This quickly changes to green, then to yellow and finally to a yellowish-red. The test is directly applicable to all body fluids except bile and whole blood, owing to the color possessed by these fluids.

*Phloroglucin Test for Free Formaldehyd.*—This was first described by Jorissen.<sup>6</sup> The reagent used consists of phloroglucin (Reagent-Merck) 0.1 gm. dissolved in 10 c.c. of 10 to 20 per cent. sodium hydroxid. When first prepared the solution acquires a bluish-violet color, but on standing becomes entirely colorless or with at most a yellowish tinge. The reagent may be used freshly prepared since the violet color does not interfere with the red of formaldehyd. The test is performed in the cold or at ordinary room temperature by the direct addition of about 0.5 c.c. of the reagent to about 1 to 2 c.c. of the fluid containing formaldehyd. A deep bright red appears instantaneously with higher concentrations of formaldehyd, but with lower concentrations of formaldehyd it requires about one-half to one minute for the color to reach its maximum intensity. The color persists for at least five minutes with dilute solutions and much longer with concentrated formaldehyd solutions.<sup>7</sup> The test is directly applicable to all body fluids except whole blood and bile. The hemoglobin of fluids containing a trace of blood (enough to give a red tint) is immediately reduced by the alkali of the reagent, the solution assuming a yellowish color, and does not interfere with the formaldehyd reaction. The reagent added to water alone gives a clear colorless solution.

*Delicacy of the Formaldehyd Tests.*—This was tested out in the following manner: Known dilutions of formaldehyd<sup>7</sup> ranging from 1:1,000 to 1:15,000,000 were made in light amber-colored urine and in water. The tests were then performed in the manner described above. The results obtained (Table 1) were as

3. Rimini: Ann. di. Farmacol. 1897, xvii, 97.

4. Arnold and Mentzel: Ztschr. Nahr. Genussm., 1902, v, 353.

5. In place of the nitroprussid, sodium ferrieyanid may be used, in which case the resulting color is red.

6. Jorissen: Bull. soc. chim. Belg., 1897-98: xi; xii, 211.

7. The concentrations of formaldehyd throughout the paper refer to absolute formaldehyd and not to the commercial (40 per cent.) solution.

follows: With the phenylhydrazin, shades of blue to green with the higher concentrations to a greenish-yellow with a concentration of 1:2,000,000, indistinguishable from that of the test plus water. The limit of delicacy obtained was 1:1,000,000. With the phloroglucin test a fine gradation of red colors was obtained, ranging from a deep bright red with the higher concentrations to a visible trace of pink in 1:10,000,000. The results with urine were similar.

## RELATIVE USEFULNESS OF THE TWO TESTS

Certain objections have been raised to the use of delicate tests for formaldehyd in clinical practice. Burnam\* has proposed the use of phenylhydrazin test as a practical clinical test owing to its lesser delicacy.

TABLE 1.—DELICACY OF FORMALDEHYD TESTS\*

Concentration of Formaldehyd	Phenylhydrazin Test	Phloroglucin Test
1:1,000	+ Deep blue	+ Very deep red
1:10,000	+ Blue	+ Less deep red
1:50,000	+ Bluish green	+ Lesser deep red
1:100,000	+ Green	+ Deep red
1:250,000	+ Green	+ Lighter deep red
1:500,000	+ Light Green	+ Light red
1:1,000,000	+ Very light green	+ Lighter red
1:2,000,000	+ (?) Very light greenish	+ Very light red
1:4,000,000	+ (?) Very light greenish	+ Light pink
1:8,000,000	+ (?) Very light greenish	+ Very light pink
1:10,000,000	+ (?) Very light greenish	+ Visible trace of pink
1:15,000,000	+ (?) Very light greenish	— Colorless
Distilled water	+ (?) Very light greenish	— Clear; colorless

\* In this table the plus sign (+) means that the test was positive; the minus sign (—) means that the test was negative.

thus serving as a reliable index of therapeutically efficient quantities of excreted formaldehyd. We have found this test to be comparatively delicate (1:1,000,000) and capable of detecting concentrations of formaldehyd below the margin of bactericidal efficiency. The phenylhydrazin test requires three reagents, two of which are unstable; and the several changes of color in urine add an element of complexity. On the

8. Burnam: THE ARCHIVES INT. MED., 1912, x, 324.

other hand, the phloroglucin test gives a constant red color with gradations of intensity depending on the concentration of formaldehyd; its application is exceedingly simple; it requires only one reagent, and for these reasons alone should be preferred to the phenylhydrazin as a more practical test. These conclusions have been reached from an extensive application of both tests simultaneously in the greater portion of the experiments embodied in this paper, as well as on other occasions.

#### CONCLUSION

The phloroglucin test for free formaldehyd (limit 1:10,000,000) is more sensitive than the phenylhydrazin (limit 1:1,000,000); it is simpler and does not give with urine the complexity of colors which is so troublesome with the phenylhydrazin test.

#### *II. Acid Facilitates, Alkali Inhibits the Liberation of Formaldehyd from Hexamethylenamin in Body Fluids*

The comparative ease with which hexamethylenamin decomposes in aqueous acid solution and its lack of decomposition in aqueous alkaline solutions might conceivably be somewhat modified in body fluids, for instance, by ferments. In the experiments which follow, various body fluids and water were used, and the effects of reaction, time and temperature were studied.

Different body fluids and water were mixed with hexamethylenamin, 0.1 and 0.5 per cent., the reaction made acid by the addition of 0.2 per cent. hydrochloric acid; or alkaline by 0.2 per cent. sodium carbonate or 0.1 per cent. ammonium hydroxid. The mixture was divided into three sets of duplicate tubes of 5 c.c. each. The phloroglucin and phenylhydrazin tests were applied (1) to one pair of tubes as soon as the dilutions were made; (2) to a second portion after the solutions were raised to the boiling point, and (3) to a third portion after the solutions were incubated for one and five hours at 37.5 C. (99.5 F.). The data obtained have been placed in Table 2. The results with both the formaldehyd tests were identical, and they have been included under one column. The doubtful experiments marked (?) in the table can probably be considered negative in the light of our further experience.

#### COMMENT ON TABLE 2

*Freshly-Made Solutions.*—Formaldehyd was liberated at once in all the acidulated fluids except in the cat's serum in which the acid was probably neutralized; this gave the test on standing. In the fluids alone, formaldehyd could be detected only in infected ascitic fluid and the stronger pancreatic extract. The composition of the ascitic fluid was unknown and it may have contained substances giving rise to true acidity

TABLE 2.—LIBERATION OF FORMALDEHYD FROM HEXAMETHYLENAMIN IN VARIOUS BODY FLUIDS \*

Hexamethylenamin 0.5 Per Cent. in	Freshly Made Solutions			Solutions Raised to the Boiling Point				Solutions Incubated for Five Hours at 37.5 C.				
	Fluid alone	Fluid Containing			Fluid alone	Fluid Con- taining 0.2 Pct. HCl	Fluid with 0.2 Pct. Na <sub>2</sub> CO <sub>3</sub>	Fluid with 0.1 Pct. NH <sub>4</sub> OH	Fluid alone	Fluid with 0.2 Pct. HCl	Fluid with 0.2 Pct. Na <sub>2</sub> CO <sub>3</sub>	Fluid with 0.1 Pct. NH <sub>4</sub> OH
		0.2 Pct. HCl	0.2 Pct. Na <sub>2</sub> CO <sub>3</sub>	0.1 Pct. Ammo- nium Hy- droxid NH <sub>4</sub> OH								
Water .....	—	+	—	...	+	+	+	...	+	+	—	...
Sodium chlorid 5 per cent.....	—	+	—	...	+	+	+	...	+	+	+	...
Urine .....	—	+	—	...	+	+	+	...	+	+	+	...
Extract cat's pan- creas (50 per cent. glycerol) .....	—†	+	—	...	+	+	+	...	+	+	+	...
Cat's serum.....	—	+	—	...	+	+	+	...	+	+	+	...
Egg albumin sat'd..	—	+	—	...	+	+	+	...	+	+	+	...
Asciatic fluid (sterile) .....	—	+	—	...	+	+	+	...	+	+	—	...
Asciatic fluid (infected) .....	+	+	—	...	+	+	+	...	+	+	—	...
Fluid of mesenteric cyst ‡ .....	—	+	+	...	+	+	+	...	+	+	+	...
Hexamethylenamin 0.1 per cent. in...	..	..	..	...	..	..	..	...	..	..	+	...
Water .....	—	+	—	—	+	+	+	—	+	+	+	—
Sodium chlorid 2 per cent.....	—	+	—	—	+	+	+	—	+	+	+	+
Urine .....	—	+	—	—	+	+	+	—	+	+	+	+
Egg albumin, sat'd.	—	+	—	—	+	+	+	—	+	+	+	+
Pancreatic extract..	+	+	—	—	+	+	+	—	+	+	+	+
Cerebrospinal fluid §	—	+	—	—	+	+	+	—	+	+	+	+

\* In the table + = present; — = absent; + ? = doubtful or slight; ± = one test positive, the other negative.  
† Stood fifteen minutes.

‡ Standing over night showed trace of formaldehyd by the phloroglucin test, none by the phenylhydrazin test.  
§ Standing for two hours showed a trace of formaldehyd by the phloroglucin test.

\*\* On standing over night, then heated, both tests showed the presence of formaldehyd.

‡ Examination of this fluid by Dr. Pilcher showed a thick, creamy emulsion; slightly alkaline to litmus; fat, 25.2 per cent. globulin and albumin present; trace of albumose; trace of dextrose; became rancid on standing.

§ Represents a collection from several individuals with different clinical conditions.



(not litmus acidity). No explanation can be offered for the behavior of the pancreatic extract. Formaldehyd was absent in practically all of the alkaline ( $\text{Na}_2\text{CO}_3$  and  $\text{NH}_4\text{OH}$ ) fluids.

*Solutions Raised to the Boiling Point.*—The data indicate the uniform presence of formaldehyd in acid fluids as well as in the fluids alone. Boiling liberates formaldehyd from all the fluids except when alkali had been added. The majority of alkaline fluids were negative, but a few appeared positive after boiling. The reactions of these fluids had not been observed, however, and this may explain the discrepancy.

*Incubation for Periods of One Hour and Five Hours at 37.5 C.*—The results for the different periods of incubation were the same and about identical with those when the solutions were raised to the boiling point. That is, formaldehyd was uniformly present in the fluids alone as well as in the acidified fluids, and absent in all of the alkaline fluids. This in general indicates the ease with which hexamethylenamin decomposes even at a moderate temperature.

#### CONCLUSION

Solutions of hexamethylenamin in water and the various body fluids used do not liberate formaldehyd immediately, but do so on boiling, or on standing one hour at 37.5 C., or at once if acid is added. Alkalies prevent the liberation, except in a few doubtful instances.

#### III. Formaldehyd Liberation and Culture Experiments with Hexamethylenamin in Pathological Fluids

The experiments hereafter related were made to determine directly the antiseptic effects of various proportions of hexamethylenamin in body fluids, and to observe whether the antiseptic effects go parallel with liberation of formaldehyd. This direct determination seemed worth while, because the higher protein content, etc., of such fluids might conceivably modify theoretical effects. The culture experiments were made by Dr. H. O. Ruh, Lakeside Hospital, as follows:

With each pathological fluid a series of tubes was prepared, each containing 7 c.c. of fluid, with different amounts of hexamethylenamin or formaldehyd. To each tube was added 1 c.c. of a very dilute suspension of bacteria (pure cultures of the different varieties). The tubes were then shaken and placed in an incubator at 37.5 C. At the end of twenty-four and forty-eight hours the character of the bacterial growth was determined by direct inspection. Turbidity indicated bacterial growth; a clear fluid indicated no growth. Some of the tubes showed differing grades of turbidity, but as the cultures were not constant in this respect no differentiation was attempted between them. In some cases the original fluids were so turbid that plate cultures were made. Here the presence of colonies indicated bacterial growth; no colonies meant no growth. The phenylhydrazin and phloroglucin tests were applied to each tube at the end of the incubation period. The results of our experiments have been placed in Table 3. The results of the formaldehyd tests were identical and have been placed in a single column.

TABLE 3.—FORMALDEHYD LIBERATION AND CULTURE EXPERIMENTS WITH HEXAMETHYLENAMIN IN PATHOLOGICAL FLUIDS\*

Fluid and Micro-Organism																		
Hexa- methyl- enamin	Bouillon (alone)		Pleural Transudate† with M. Staphyl- ococcus		Pleural Transudate‡ with B. Typhosus		Ascitic Fluid§ With B. Typhosus		Hydrocoele Fluid** with M. Staphyl- ococcus		Ascitic Fluid¶ with B. Pyocyanus		Ascitic Fluid (sterile) with B. Typhosus		Chest Fluid (alone)		Bouillon with M. Staphyl- ococcus	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1:200	....	....	....	....	....	....	....	....	++	++	++	++	++	++	++	++	++	++
1:300	....	....	....	....	....	....	....	....	++	++	++	++	++	++	++	++	++	++
1:500	....	—	....	++	....	++	....	++	++	++	++	++	++	++	++	++	++	++
1:1,000	....	—	....	++	....	++	....	++	++	++	++	++	++	++	++	++	++	++
1:2,000	....	—	....	....	....	?	....	?	++	++	++	++	++	++	++	++	++	++
1:5,000	....	—	....	++	....	++	....	++	++	++	++	++	++	++	++	++	++	++
1:10,000	....	—	....	++	....	++	....	++	++	++	++	++	++	++	++	++	++	++
1:50,000	....	—	....	++	....	++	....	++	++	++	++	++	++	++	++	++	++	++
Formal- dehyd	Bouillon with B. Staphylo- coccus																	
	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++
1:1,000	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++
1:2,000	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++
1:5,000	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++
1:10,000	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++
1:20,000	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++
1:40,000	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++
1:80,000	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++	—	++

\* B = bacteria; F = formaldehyd; + = present; — = absent.

† Pleural transudate from a patient with valvular incompetency and synecchia cordis. The fluid was slightly cloudy and contained a few erythrocytes, therefore plate cultures were made.

‡ Pleural transudate from a patient with valvular disease. The fluid was very slightly turbid.

§ Ascitic fluid from a case of hepatic cirrhosis. The fluid was very slightly turbid. Slight increase in cloudiness produced by bacterial growth could easily be made out.

\*\* The fluid was clear, colorless, and sterile.

¶ Ascitic fluid from a case of hepatic cirrhosis. The fluid was slightly turbid and contained a few erythrocytes. Plate cultures were made.

Ten per cent. dilutions of sterile bile in 0.9 per cent. sodium chlorid were incubated with *B. typhosus*, *M. staphylococcus aureus*, and *B. pyocyaneus* in tubes as well as in plates, but the results were unsatisfactory on account of the darkness and turbidity of the media.

TABLE 5.—BACTERICIDAL PROPER

Micro-Organism	Culture Medium	Character of Bacterial Growth and Presence		
		1:50	1:100	1:200
<i>M. pyogenes aureus</i>	Agar alone	None (1), (2) +	×	.....
<i>M. ureae</i>	Agar alone	None (1) +	×	.....
<i>B. diphtheriae</i>	Agar alone	no colonies (1) + strong	No colonies (1) + strong	.....
<i>B. typhosus</i>	0.2 per cent. CO <sub>2</sub> -agar	.....	Diffuse (1) (2) —	×
<i>B. typhosus</i>	0.2 per cent. CO <sub>2</sub> -agar	None (1) 10 colonies (2) +	None (1) Diffuse (2) +?	1 colony (1) 6 colonies (2) —
<i>B. typhosus</i>	0.5 per cent. NH <sub>3</sub> -agar	None (1), (2) + trace	None (1), (2) —	×
<i>B. typhosus</i>	0.5 per cent. CO <sub>2</sub> -agar	None (1) several small colonies (2) —	None (1) Several colonies (2) —	×
<i>M. pyogenes aureus</i>	0.5 per cent. CO <sub>2</sub> -agar	diffuse (1) (2) —	×	×
<i>M. pyogenes aureus</i>	0.5 per cent. NH <sub>3</sub> -agar	None (1) diffuse (2) +	None (1) Diffuse (2) —	×
<i>M. pyogenes aureus</i>	0.5 per cent. NH <sub>3</sub> -agar	diffuse (1) +	Diffuse (1) —	×
<i>M. pyogenes aureus</i>	0.5 per cent. NH <sub>3</sub> -agar	Numerous colonies (1), (2) +?	Numerous colonies (1), (2) —	.....
<i>B. coli</i>	0.5 per cent. NH <sub>3</sub> -agar	Many colonies (1) + slight	Many colonies (1) —	.....
<i>B. pyocyaneus</i>	0.5 per cent. NH <sub>3</sub> -agar	Very marked growth (1) +?	Very marked growth (1) —	.....
<i>B. coli</i>	0.5 per cent. NH <sub>3</sub> -agar	None (1) + trace	Marked growth (1) —	.....
<i>Actinomyces</i>	0.5 per cent. NH <sub>3</sub> -agar	12 colonies (1) + trace	12 colonies (1) —	.....
<i>M. ureae</i>	0.5 per cent. NH <sub>3</sub> -agar	Numerous colonies (1) +?	Numerous colonies (1) —	.....
<i>B. pneumoniae</i>	0.5 per cent. NH <sub>3</sub> -agar	Numerous colonies (1) +	Numerous colonies (1) —	.....
<i>B. anthrax</i>	0.5 per cent. NH <sub>3</sub> -agar	Fewer colonies than rest (1) +	Great many colonies (1) —	.....
<i>B. diphtheriae</i>	0.5 per cent. NH <sub>3</sub> -agar	Diffuse cloudiness (1) (2) +?	Diffuse colonies (1) (2) —	.....
<i>B. diphtheriae</i>	0.5 per cent. NH <sub>3</sub> -agar	diffuse (1) +	Diffuse (1) —	.....

\* CO<sub>2</sub>-agar refers to agar modified with sodium carbonate; NH<sub>3</sub>-agar refers "×" are the same as the next higher concentration.

(1), growth at end of twenty-four hours; (2), at end of forty-eight hours;

of Formaldehyd in Hexamethylenamin			Remarks
1:250	1:500	Blank	
Numerous colonies (1), (2) + ×	×	Numerous colonies (1), (2) — Numerous colonies (1) — Diffuse growth (1) + ×	
88 colonies (1) + ×	162 colonies (1) + ×	×	Growth diffuse in all plates at end of seventy-two hours.
Diffuse (1) 5 colonies (2) — ×	Diffuse (1), (2) — ×	×	Experiment repeated; no growth in any of the plates. Colonies about equally distributed in all plates.
×	×	×	At end of forty-eight and sixty-eight hours growths more diffuse.
Diffuse (1), (2) — ×	×	×	At end of seventy-two hours growth increased in all plates.
×	×	×	
Considerable growth (1) — ×	×	×	
×	×	×	
30 colonies (1) — ×	50 colonies (1) — ×	28 colonies (1) — ×	
×	×	×	
×	×	×	
×	×	×	Growth at end of ninety-six hours same; formaldehyd tests same. Involution form of bacterium used.
×	×	×	Distribution of colonies same in all plates. Fresh culture used.

to agar modified with ammonium hydroxid. The description of those marked

+, formaldehyd present; —, formaldehyd absent.



## COMMENT ON TABLE 3

*Hexamethylenamin.*—Bacterial growth was not prevented in any of these fluids even in solutions containing 0.5 per cent. of hexamethylenamin, a proportion much higher than could obtain anywhere in the body. It is therefore improbable that the administration of hexamethylenamin could have marked antiseptic effects in the tissues, although a partial restraining action is not positively excluded. Traces of formaldehyd were liberated, but not enough to be effective; i. e., it must have been less than 1:10,000.

*Formaldehyd.*—The limit of bactericidal efficiency in general is from 1:10,000 to 1:5,000 for a majority of the organisms. In the sterile ascitic fluid which was incubated with *B. typhosus*, bacteria and formaldehyd were simultaneously present in all concentrations, while in the experiment which consisted of ascitic fluid and *B. typhosus* no bacteria grew in any of the concentrations, although formaldehyd was present in all the cultures. No explanation can be offered for the results in these two experiments.

*IV. Hexamethylenamin Itself is Not Bactericidal*

The experiments just described indicate that the bactericidal efficiency of hexamethylenamin depends on the liberated formaldehyd. This was also the conclusion of Jordan,<sup>9</sup> who observed that the hexamethylenamin prevented putrefaction of urine and the growth of staphylococci when the formaldehyd concentration, as estimated by the phloroglucin test, corresponded to about 1:10,000; i. e., the same concentration in which pure formaldehyd is bactericidal. In alkaline urines putrefaction ensued more rapidly and staphylococci grew readily. This is indirect evidence that the antiseptic properties of hexamethylenamin must depend on the liberated formaldehyd. Others have made similar statements, but without offering decisive evidence that hexamethylenamin does not possess bactericidal properties.

The question was decided definitely in the negative by incubation experiments with several species of bacteria in hexamethylenamin media in which the liberation of formaldehyd was prevented. This medium consisted of agar modified with sodium carbonate, or with ammonium hydroxid. In this way an alkaline medium was secured which prevented the liberation of formaldehyd and allowed the hexamethylenamin to act as such on the bacteria.<sup>10</sup> All of the cultures consisted of plates. Different concentrations of hexamethylenamin were used, but the total volume of the culture in each case was always the same. The same volume of a

9. Jordan: *Biochem. Jour.*, 1911, v, 274.

10. We wish to express our thanks to Professor Perkins, of the Laboratory of Hygiene and Preventive Medicine, who supplied us with pure cultures.

dilute culture of the bacteria was used in each plate. At the end of the incubation period, a small portion of the culture material was excised, treated with a little (5 c.c.) distilled water and tested with the phenylhydrazin and phloroglucin tests for formaldehyd.

The details of a typical experiment are as follows:

*Experiment 9.*—*B. coli*, bacterial emulsion consisting of three loops of a pure culture in 3 c.c. of bouillon; hexamethylenamin, 10 per cent. solution in water; ammonium hydroxid, 0.5 per cent. solution of the concentrated ammonium hydroxid (sp. gr. 0.9); and plain agar melted on a water-bath so as just to flow. The solutions were mixed in the order given in Table 4.

TABLE 4.—EXPERIMENT WITH COLON BACTERIA. RESULT\*

Hexamethyl- enamin, c.c.	Concentra- tion	Plain Agar† c.c.	Phloro- glucin‡	Phenyl- hydrazin‡
1.0	1:50	8.3	+ sl	+ sl
0.5	1:100	8.8	—	—
0.2	1:250	9.1	—	—
0.1	1:500	9.2	—	—
0.0	:0	9.3	—	—

\* Numerous colonies appeared at the end of twenty-four hours in all of the plates.

† Preceding the plain agar 0.5 c.c. ammonium hydroxid and 0.2 c.c. bacteria were added to the hexamethylenamin in the order named.

‡ Formaldehyd; + present; — absent; sl. = slight.

All plates were in duplicate. The mixing of the contents was accomplished by quickly and gently tilting and rotating each plate before the agar solidified. Then they were placed in an incubator at 37.5 C. At the end of twenty-four hours, and in some cases at the end of forty-eight hours, the plates were inspected and the formaldehyd tests applied.

The results of the experiments have been placed in Table 5.

#### COMMENT ON TABLE 5

The data from the first three experiments in which no alkali was added and in which liberation of formaldehyd took place, show that no bacterial growth occurred in concentrations of 1:100 and 1:50 of hexamethylenamin. In the lower concentrations bacteria grew about as effectively as in plates containing no hexamethylenamin. In these low concentrations of hexamethylenamin not enough formaldehyd was liberated to inhibit the growth of bacteria. The presence of formaldehyd in the plates was confirmed by the tests.

On the other hand, in agar modified with ammonium hydroxid and sodium carbonate, and which did not contain liberated formaldehyd, the growth of bacteria took place uniformly throughout all of the plates, even those containing 2 per cent. of hexamethylenamin. Usually the growths

were so diffuse that it was impossible to estimate the number of colonies present and they differed but slightly, if at all, from those of the blanks (consisting of the special agar minus hexamethylenamin). In a majority of the plates containing concentrations of 1:50 hexamethylenamin not quite enough alkali was present to inhibit completely the liberation of formaldehyd. Traces of formaldehyd could be detected, but not enough was present to affect the bacterial growth. The experiments with different species of organisms, some highly resistant and some susceptible to formaldehyd, yielded practically identical results. These results stand out as a convincing proof that hexamethylenamin in itself is not bactericidal, but that the bactericidal properties depend on the liberation of formaldehyd.

*V. The Liberation of Formaldehyd from Hexamethylenamin Depends on the Hydrogen Ion Concentration of the Solution*

Since the bactericidal properties of hexamethylenamin depend on the liberated formaldehyd, it is essential to ascertain the factors which determine this liberation under biological conditions. The possible factors are acidity, temperature, time, concentration, ferments and perhaps others. Of these, as has been previously remarked, the acidity of the fluid is of the greatest importance. Our results indicated that the liberation could occur with degrees of acidity lower than those appreciated by litmus, and closely approximating to that of certain body fluids. On the other hand, no decomposition whatsoever occurred in solutions rendered alkaline by 0.2 per cent. sodium carbonate, a degree of alkalinity frequently exceeded by certain body fluids, particularly in the gastrointestinal tract. Therefore, we resorted to the determination of the true reactions of fluids by the use of the indicator methods elaborated by Sørensen,<sup>11</sup> and later extended by L. J. Henderson<sup>12</sup> and others, to the various body fluids.

*True Reaction.*—Before passing on to the experiments themselves, it is necessary to say something concerning the principles on which our knowledge of the true reaction of a fluid is based. Briefly, the true reaction of a solution in terms of physical chemistry depends on the relative concentrations of the hydrogen (H) and hydroxyl (OH) ions therein. When the concentration of the hydrogen and hydroxyl ions are equal the condition is spoken of as neutrality. When there is a preponderance of hydrogen (H) ions true acidity exists, while a preponderance of hydroxyl (OH) ions means true alkalinity. The hydrogen may come from an acid such as hydrochloric acid (HCl) or an acid salt such as monosodium phosphate ( $\text{Na H}_2\text{PO}_4$ ). In either case it is only the hydrogen which is ionized or dissociated that is pertinent to the acid reaction of a solution. These ions possess certain electric charges, for when water or electrolytes (salts) are decomposed electrolytically the acid ions aggregate at one pole, while the basic

11. Sørensen: *Ergebnisse d. Physiol.*, 1912, xii, 394.

12. Henderson, L. J.: *ibid.*, 1909, viii, 254; *ibid.*, *Jour. Biol. Chem.*, 1911, ix, 403.

ions aggregate at the other pole. Thus hydrogen ions carry a positive charge and aggregate at the negative pole. The mode of expression commonly used is  $^+(\text{H})$ ; for hydroxyl ( $^-\text{OH}$ .)

The reaction of a solution can be given a quantitative expression by measuring the values of  $^+(\text{H})$  and  $^-\text{OH}$  (L. J. Henderson<sup>13</sup>). This is highly essential for the accurate study of the relation of reactions to different chemical phenomena. First, the point of neutrality must be known. It has been determined experimentally that at 25 C. the concentrations of  $^+(\text{H})$  is 0.000,000,1 gm., and that of  $^-\text{OH}$  is 0.000,0017 gm. in 1,000 gm. of water. Dividing this by the atomic weights, it is seen that the number of  $^+(\text{H})$  and  $^-\text{OH}$  ions is alike, as demanded by theory. Expressed logarithmically the figure for the ionized hydrogen ( $^+(\text{H})$ ) would read  $^+\text{H}^{10-7}$  as the quantitative expression of neutrality. For convenience the exponential digit alone without the minus sign is now used, that is, instead of  $^+(\text{H}^{10-7})$ , merely 7.0 is written. We have used this mode of expression in our paper. A number of actual  $^+(\text{H})$  determinations will serve to illustrate just what is meant by alkalinity and acidity:

Lowest $^+(\text{H})$ concentration =	7.3	} true alkalinity.
	7.2	
	7.1	
	7.0	} = neutrality.
	6.9	
Highest $^+(\text{H})$ concentration =	6.8	} true acidity.
	6.7	
	6.6	
	6.5	

The method now commonly used for the determination of the  $^+(\text{H})$  concentration of fluids is that devised and described by Sørensen. The objections to this method raised by Rona<sup>15</sup> do not concern us here. The method of Sørensen consists essentially of a series of indicators which produce certain changes of

color with certain definite range of the  $^+(\text{H})$  concentration. Sets of standards with known ranges of  $^+(\text{H})$  concentrations containing the different indicators are used for comparison with the unknown solution. In detail it consists of running 5 c.c. of the fluid, for instance, urine, in a bottle containing the indicator. This is then diluted to 125 c.c. with distilled water freshly boiled (to remove carbon dioxide) and previously cooled to a volume equal to that of the standard containing an equal volume of the indicator. The comparison is then made until a standard is found whose color exactly matches that of the unknown.

The  $^+(\text{H})$  concentration of the matched standard is the  $^+(\text{H})$  concentration of the fluid. Certain precautions must be exercised in the selection of a practically neutral water for the dilution of fluids, and glassware must not be contaminated with bases or acids. Turbidity in the diluted solutions must be avoided. In such cases a smaller volume of fluid is used, as it has recently been

13. Henderson, L. J.: Science, 1913, xxxvii, 389.

15. Rona: Handbuch d. Biochemischen Arbeitsmethoden, 1911, v, 317.



shown by L. J. Henderson and Palmer<sup>16</sup> that dilution does not affect the determination of the  $(\overset{+}{H})$  concentration to any appreciable extent.

*Relation Between Formaldehyd Liberation and Hydrogen Ion Concentrations in Urines*

We have examined<sup>17</sup> urines, cerebrospinal fluids, bloods, gastric juice and a few pathological exudates from persons receiving hexamethylenamin, and other fluids chiefly from dogs. In this section the discussion will be limited to human urines, since they exhibit the most variable concentrations of ionizable hydrogen  $(\overset{+}{H})$ . The remaining fluids will be taken up in the sections to follow.

The following tabulation is based on 292 hexamethylenamin urines, some freshly voided, others that were standing. (The influence of standing will be discussed later.) The freshly voided urines consisted of fractional specimens which were obtained as often as the individuals (dispensary patients<sup>18</sup> and laboratory assistants) could micturate. Standing urines represented collections of twenty-four hours in the majority of cases. These were obtained chiefly from patients in the wards. The dosage of hexamethylenamin varied, but enough was usually excreted in the urine so that liberation of formaldehyd could take place. The phloroglucin test for formaldehyd was used. The data obtained have been placed into Table 6. In this, the urines are arranged in the order of their  $(\overset{+}{H})$  concentrations.

COMMENT ON TABLE 6

It is readily seen from Table 6 that free formaldehyd was present in the majority, or 221 (97.4 per cent.) of the urines which were acid; that is,  $(\overset{+}{H})$  concentration over 7.0 (from 6.98 to 4.48). Of the six urines which were doubtful, three showed comparatively high acidities. This is ascribed to contamination of our receptacles, as judged from the reactions of the adjoining samples from the individuals, and from the fact that lower acidities generally showed the presence of formaldehyd. The remaining doubtful specimens possess acidities which are close to the neutral point, and these may have been contaminated or contain portions of alkaline fractions which reduced the intensity of the formaldehyd reactions.

Of 59 urines which exhibited alkalinity, that is  $(\overset{+}{H})$  concentration of less than 7.0 (from 7.1 to 7.4), the majority or 57 (about 96.8 per

16. Henderson, L. J., and Palmer: Jour. Biol. Chem., 1913, xiii, 393.

17. The acidity determinations were made under the supervision of Professor Haskins of the Bio-Chemical Laboratory.

18. From the Medical Dispensary, Western Reserve University and Lakeside hospital.

cent.) show the absence of formaldehyd. The two urines which are marked doubtful may have contained portions of preceding acid urine fractions, and which would therefore contain traces of formaldehyd.

TABLE 6.—RELATION OF HYDROGEN ION CONCENTRATION OF HEXAMETHYLENAMIN URINES TO LIBERATION OF FORMALDEHYD

	$\begin{matrix} + \\ (H) \end{matrix}^*$	Total No. of Hexamethylenamin Urines with Formaldehyd			$\begin{matrix} + \\ (H) \end{matrix}^*$	Total No. of Hexamethylenamin Urines with Formaldehyd	
		Present	Absent			Present	Absent
$\begin{matrix} \nearrow \\ \text{Increasing} \\ \text{alkalinity} \\ \text{Neutrality} \rightarrow \\ \searrow \\ \text{Increasing} \\ \text{acidity} \end{matrix}$	7.4 ..	....	5	$\begin{matrix} \text{Increasing} \\ \text{acidity} \\ \downarrow \end{matrix}$	6.25 ..	2	....
	7.3 ..	....	8		6.24 ..	1	....
	7.25 ..	....	3		6.2 ..	9	....
	7.2 ..	....	29		6.15 ..	1	....
	7.16 ..	1	4		6.1 ..	5	....
	7.15 ..	....	2		6.0 ..	6	....
	7.1 ..	1 (?)	6		5.95 ..	26	....
	7.0 ..	1 (?)	5		5.9 ..	2	....
	6.98 ..	....	1		5.85 ..	6	....
	6.95 ..	4	1		5.82 ..	1	....
	6.94 ..	....	1		5.8 ..	8	....
	6.92 ..	1 (?)	....		5.75 ..	5	....
	6.9 ..	3	1		5.7 ..	3	....
	6.89 ..	1	....		5.65 ..	5	....
	6.85 ..	3	....		5.6 ..	4	....
	6.75 ..	2	....		5.58 ..	1	....
	6.72 ..	1	....		5.55 ..	1	....
	6.7 ..	10	1 (?)		5.5 ..	3	....
	6.65 ..	1	....		5.47 ..	1	....
	6.64 ..	3	....		5.45 ..	1	....
	6.6 ..	7	....		5.4 ..	4	....
	6.55 ..	1	....		5.35 ..	1	....
	6.52 ..	1	....		5.3 ..	3	....
	6.5 ..	10	....		5.25 ..	1	....
	6.47 ..	4	....		5.2 ..	3	....
	6.45 ..	6	....		5.19 ..	6	....
	6.4 ..	1	....		5.1 ..	10	....
	6.38 ..	2	....		5.0 ..	16	1
	6.37 ..	1	....		4.85 ..	1	....
	6.35 ..	9	....		4.48 ..	1	....
	6.32 ..	1	....				
	6.3 ..	12	....		Total .	224	68

\*  $\begin{matrix} + \\ (H) \end{matrix}$  refers to hydrogen ion concentration.

Of 6 urines which exhibited neutrality, that is,  $\begin{matrix} + \\ (H) \end{matrix}$  concentration of 7.0, the majority or 5 (83 per cent.) showed the absence of formaldehyd. Here again the single doubtful specimen may have contained a portion of previously used acid urine.

It was observed that many of the first, fractional urines (either acid or alkaline), which contained only a trace (more or less) of hexamethyl-

enamin as indicated by the bromin-water test, did not show detectable traces of formaldehyd. It is possible that not enough hexamethylenamin was present to liberate sufficient formaldehyd for the phloroglucin test. We have, therefore, omitted from the table the first fraction of urines voided.

#### CONCLUSIONS

Practically all hexamethylenamin urines which exhibit true acidity, that is,  $(\overset{+}{H})$  concentration over 7.0, show the presence of formaldehyd; while urines which exhibit true alkalinity, that is,  $(\overset{+}{H})$  concentration less than 7.0, do not show detectable traces of formaldehyd.<sup>19</sup> The very rare exceptions to this rule are probably technical errors.

#### VI. Previous Investigations on the Distribution, Excretion and Decomposition of Hexamethylenamin in the Body Fluids

It might be objected that the behavior of hexamethylenamin in body fluids *in vitro* under different conditions might be rather different than its behavior in the fluids of the living organism; and the question deserves to be investigated directly. In the preceding sections it was shown that the bactericidal properties of hexamethylenamin depend on the liberated formaldehyd, and that formaldehyd liberation can take place only in a truly acid medium. It is, therefore, important to determine directly whether hexamethylenamin is present in various tissues, fluids, etc., as such or as formaldehyd, and to note the true reaction of the various body fluids as well.

It has been shown by Crowe<sup>20</sup> that hexamethylenamin is excreted in the bile, pancreatic juice, cerebrospinal fluid and synovial fluid of human beings and in the saliva and milk of dogs; he supposed, but without any direct evidence, that it liberates formaldehyd in these situations. Flexner and Clark<sup>21</sup> and Hald<sup>22</sup> have reported its excretion in the cerebrospinal fluids of monkeys and patients ill with anterior poliomyelitis and meningitis, respectively. Bucura,<sup>23</sup> Rieder,<sup>24</sup> and Schmid and Schröter<sup>25</sup> have shown that free hexamethylenamin, but no free formaldehyd is excreted in human milk. Hanzlik<sup>26</sup> has shown that hexamethylenamin, but no free formaldehyd is excreted in human saliva. This has

19. During the course of our work a paper has appeared by G. W. Smith:

Boston Med. and Surg. Jour., 1913, clxviii, 713, on a study of the  $(\overset{+}{H})$  concentration in hexamethylenamin urines. Smith's results are practically identical with those obtained by us.

20. Crowe: Johns Hopkins Bull., 1908, xix, 109; *ibid.*, xx, 102; Arch. intern. pharmacodyn., 1908, xviii, 315.

21. Flexner and Clark: Jour. Am. Med. Assn., 1911, lvi, 585.

22. Hald: Arch. f. exper. Path. u. Pharmakol., 1911, lxiv, 329.

23. Bucura: Arch. exper. Path. Therap., 1907, iv, 398.

24. Rieder: Monatschr. Kinderheilk., xi, 80; Chem. Abstracts, 1913, vii, 1057.

25. Schmid and Schröter: Cent. f. Physiol. u. Path. d. Stoffwechsel, 1910, v, 129.

26. Hanzlik: Jour. Am. Med. Assn., 1910, liv, 1940.

been confirmed by Zák,<sup>27</sup> who also detected hexamethylenamin in the bronchial secretion of a tuberculous patient: this has also been shown in sputa of tuberculosis and pneumonia by Armstrong and Goodman.<sup>28</sup> The elimination of hexamethylenamin in the middle ear has been shown by W. M. Barton;<sup>29</sup> in the anterior chamber of the eye by W. S. Gradle,<sup>30</sup> and in the aqueous and vitreous humors by Whitham.<sup>31</sup> In the urine the excretion of the drug as well as formaldehyd were first reported by Nicolaier.<sup>32</sup> Since then, however, considerable confusion has existed as to the constancy of the appearance of hexamethylenamin alone or in the liberation of formaldehyd. This confusion is reflected in the current text-books. Burnam<sup>3</sup> has reported that formaldehyd may be present or absent in either acid or alkaline urines. It has been reported by L'Esperance and Cabot<sup>33</sup> that only one-half of their patients excreted formaldehyd. These observers took no note of the reaction of the urines and used the phenylhydrazin test for formaldehyd. Jenness<sup>34</sup> reports that 53 per cent. of urines of two hundred individuals showed formaldehyd. Most of these were acid to litmus and the phenylhydrazin test was used. Using the phloroglucin test for formaldehyd, Sollmann<sup>35</sup> observed that only a small portion of the hexamethylenamin appears as formaldehyd if the urine is not voided too frequently. That acidity of urine facilitates formaldehyd liberation was suggested by Wiggs.<sup>36</sup> Jordan<sup>9</sup> reports that all acid hexamethylenamin urines (litmus and titration acidities) show the presence of formaldehyd by the phloroglucin test, and that alkaline urines never show it. These reports of inconstancy of liberation of formaldehyd in hexamethylenamin urines are due essentially to the lack of appreciation of a very important factor, namely, the true reaction of the urine. The time of sojourn of the urine in the bladder is very likely a contributory factor in facilitating the liberation of formaldehyd.

Concerning the presence of free formaldehyd in other body fluids after administration of hexamethylenamin, it has been heretofore shown by one of us that the saliva does not contain it and Rieder and Schmid and Schröter have shown that human milk does not contain formaldehyd. In other fluids such as bile, cerebrospinal fluid, blood serum, synovial, pericardial and pleural fluids, vitreous and aqueous humors no data are available from the literature as to the presence of free formaldehyd. This is due largely to the character of the tests employed. That is, these tests apply both to free formaldehyd and to formaldehyd liberated by the action of sulphuric acid (or other mineral acids) on hexamethylenamin.

## VII. *Hexamethylenamin Does Not Liberate Free Formaldehyd in Body Fluids, Except when Truly Acid, Namely, Urine and Gastric Juice*

In the experiments to be described we have sought to determine whether hexamethylenamin exists as such or as free formaldehyd in the various body fluids and in freshly formed urine. This was investigated in experiments on dogs as well as in observations on fluids from human

27. Zák: Wien. klin. Wchnschr., 1912, xxv, 151.

28. Armstrong, J. J., and Goodman, E. H.: Jour. Am. Med. Assn., 1911, lvi, 1553.

29. Barton, W. M.: Jour. Am. Med. Assn., 1910, liv, 871.

30. Gradle, W. S.: Ophthalmic Record, March, 1911.

31. Whitham: Arch. Ophthalmology, 1912, xli, 604.

32. Nicolaier, A.: Ztschr. f. klin. Med., 1899, xxxviii, 350.

33. L'Esperance and Cabot: Boston Med. and Surg. Jour., clxvii, 577.

34. Jenness: Jour. Am. Med. Assn., 1913, lx, 662.

35. Sollmann: Jour. Am. Med. Assn., 1908, li, 818.

36. Wiggs: South. Med. Jour., Dec., 1910.



beings. The experiments on dogs consisted of injecting hexamethylenamin directly into a ligated loop of intestine and noting the time of the appearance of hexamethylenamin and formaldehyd in the blood and urine after short intervals of time.

All the animals received the regular laboratory anesthesia consisting of 0.02 gm. per kilo of morphin followed by ether. A tracheal cannula, a carotid cannula (for collecting samples of blood) and a vein cannula for saline injections were inserted. Laparatomy was performed; a long loop (40 cm.) of small intestine was exposed and injected with the hexamethylenamin dissolved in 100 c.c. of distilled water. From 25 to 50 c.c. of 0.9 per cent. saline were injected each time that a sample of blood was drawn in order to replace the fluid lost by repeated bleeding and to maintain diuresis throughout the experiment. The urine was collected by inserting a cannula into a ureter (usually the right) about 5 cm. above the point of entrance into the bladder. In Experiment 5 the ureteral cannula was placed as high as possible into the pelvis of the right kidney. In this way freshly formed urine was available for the tests.

Blood was collected from the left carotid artery at intervals of approximately five minutes. Each sample measured 10 to 15 c.c. This was rapidly defibrinated in a small evaporating dish, the clot of fibrin withdrawn and the remainder of the defibrinated blood was centrifugalized with a centrifuge possessing 3,000 revolutions per minute, which separates red blood-corpuscles from serum within five minutes, so that a perfectly clear and straw-colored supernatant fluid results. Approximately four minutes were required for defibrinating and five minutes for centrifugalization. Allowing one to two minutes for transfer of the serum, the entire process was completed in about ten minutes. The phloroglucin test was then applied directly to the serum. Inasmuch as the bromin-water test could not here be employed, a portion of the serum was reserved in each case, and this was distilled with a few drops of concentrated sulphuric acid and the phloroglucin test applied to the distillate.

The experiments were usually conducted until the height of hexamethylenamin excretion had been reached in the urine, as judged by the character of the precipitates with bromin-water. Then the animal was bled and finally killed with chloroform. The remaining body fluids were now obtained by carefully opening the different cavities and pipetting out the contents. Sufficient quantities of all fluids were usually obtained for the application of the tests, except cerebrospinal fluid. For free formaldehyd, the phloroglucin test was directly applied and the presence of hexamethylenamin was detected by distillation in the same manner as in blood-serum.

TABLE 7.—RESULTS OF EXAMINATION OF BODY FLUIDS IN EXPERIMENT FOUR

Blood			Urine			Other Body Fluids (post-mortem)			
Time	For- maldehyd	Hexa- methyl- enamin	Time	For- maldehyd	Hexa- methyl- enamin	Fluid	Time	For- maldehyd	Hexa- methyl- enamin
10:54	—	—*	11:02	—	—*	Pericardial	....	—	+
10:57	—	+	11:07	—	+	Pleural	....	—	....
11:12	—	+	11:15	+	+	Cerebro- spinal	....	—	+
11:17	—	+	11:20	+	+	Synovial	....	—	+
11:38	—	+	11:26	+	+	Vitreous	....	—	+
11:54	—	+	11:43	+	+	humor	....	—	+
12:26	—	+	11:49	+	+	Aqueous humor	....	?	+
.....	....	....	12:06	+	+	Bile	11:55	+	+
.....	....	....	12:32	+	+	Intestine contents	12:25	+	+
						Intestine contents			+

\* Trace. † Marked. ‡ Strong. In this table, + = present; — = absent.

The results of the tests for formaldehyd in the fluids collected at different intervals were practically identical in all of the animals. A detailed protocol of one of the experiments (Table 7) is here appended to illustrate the mode of procedure.

*Experiment 4.*—Dog weighing 8.6 kg.; 0.2 gm. hexamethylenamin per kilo (total, 1.72 gm.) dissolved in 100 c.c. water and injected into ligated loop of intestine (50 cm.) at 10:54 a. m.

Bromin water did not give a precipitate with the dog's urine before the drug was given. Secretion of urine small during first part of experiment; perhaps due to withdrawal of considerable blood. Later secretion of urine improved by injections (50 c.c. at a time) of saline. Animal killed at 12:32 p. m.

The data from all of the experiments have been concentrated into Table 8.

TABLE 8.—LIBERATION OF FORMALDEHYD FROM

Animal Experiment Number	Hexamethyl- enamin, gm. per Kilo by Intestine	Blood			Urine		
		H	Time of First Appearance After Injec- tion (mins.)	Total Time Under Ob- servation. (Mins.)	H	Time of First Appearance After Injec- tion (mins.)	Total Time Under Ob- servation. (Mins.)
		F			F		
Cat 1 .....	0.1	H +	5	25			
2.0 kg.		F —					
Dog 2 .....	0.5	H +	..	..	H +	5	69
6.0 kg.		F —	..	..	F +	10	64
Dog 3 .....	0.5	H +	5	75	H +	5	83
11.5 kg.		F —	..	75	F +	10	78
Dog 4 .....	0.2	H +	3	29	H +	13	25
8.6 kg. †		F —	..	29	F +	21	17
Dog 5 .....	0.3	H +	4	83	H +	12	75
5.6 kg. †		F —	..	83	F +	26	61
Dog 6 .....	0.3	H +	5	85	H +	9	97
8.0 kg.		F —	..	85	F +	13	88
Average .....	....	H +	4.4	..	H +	9	..
		F —	0	..	F +	16	..

† Flow of urine scanty.  
\* "H" refers to hexamethylenamin; "F" refers to free formaldehyd; + means present;

COMMENT ON TABLE 8

*Blood:* The results as shown in Table 8 indicate that hexamethylenamin appears in blood about five minutes after administration of the drug. The maximum concentration appears in about thirty minutes. Of course, this will be influenced by the rate of absorption. It is shown that free formaldehyd did not appear in the bloods of any of the animals, and our protocols show that free formaldehyd was absent in all of the samples of

blood collected. The ( $\bar{H}$ )<sup>+</sup> concentration of blood-serum is known to be never below 7.0, and it may be slightly alkaline. The bloods of several dogs which we have examined showed it to be 7.0 as a rule, that is, it is neutral. This explains the absence of formaldehyd in bloods containing hexamethylenamin.

The bloods of fourteen different patients who received 2 gm. each of hexamethylenamin before the sample was collected, showed the presence of hexamethylenamin, but no free formaldehyd. The ( $\bar{H}$ )<sup>+</sup> concentrations of their serums ranged from 7.4 to 7.15, the mean value being 7.2.

## HEXAMETHYLENAMIN IN BODY FLUIDS OF ANIMALS\*

Other Body Fluids						
Contents of Ligated Intestine	Cerebro- spinal Fluid	Pericardial Fluid	Pleural Fluid	Synovial Fluid	Aqueous Humor	Vitreous Humor
H + F +	H + F —	H + F —	H + F —	H + F —	H + F —	H + F —
H + F +	..... .....	H + F —	H + F —	H + F —	H + F —	H + F —
H + F +	H + F —	H + F —	..... F —	H + F —	H + tr F —	H + tr F —
H + F —	H + F —	H + F —	..... F —	H + F —	H + F —	H + F —
H + F +	H + F —	H + F —	H + F —	H + F —	H + F —	H + F —
..... .....	..... .....	..... .....	..... .....	..... .....	..... .....	..... .....

— means absent; "tr" means trace.

*Urine:* Table 8 indicates that hexamethylenamin appeared in the urine of dogs in approximately nine minutes after administration. The time of maximum concentration, as judged by the character of the bromin-water precipitates, varied considerably in the different individuals; but in the animals with a good continuous secretion of urine it might be said to have taken place about forty-five minutes after administration.



The average time of the appearance of formaldehyd was sixteen minutes; that is, somewhat delayed as compared with that of hexamethylenamin. All of the urinary specimens collected were acid to litmus, which means that they possessed a fairly high degree of acidity by the indicator method used for the estimation of  $(\overset{+}{H})$  concentrations. After formaldehyd made its appearance it was simultaneously present with hexamethylenamin in all of the freshly voided specimens of urine of each animal.

The greater portion of our data on urines was obtained from patients. Confined and ambulatory patients were used. In the case of the ambulatory patients, 2 gm. of hexamethylenamin were given by mouth, and the urine was collected as often as the patient could void it. The phloroglucin test for free formaldehyd was applied to the freshly voided specimens in the dispensary and the  $(\overset{+}{H})$  concentrations were determined within two to three hours; the urines being meanwhile preserved by toluol. Patients that were confined in the wards received continuous and different doses of hexamethylenamin, their urines were collected (with toluol) for periods of twenty-four hours and the tests and the acidity determinations were performed at the end of this time. In some cases the urines stood for longer periods than twenty-four hours. The data on urines have been placed in two separate tables (Tables 9 and 10), and will be treated separately.

#### COMMENT ON TABLE 9

*Freshly Voided Urines.*—Table 9 indicates that free formaldehyd was present in practically all (98.9 per cent.) of the urines which were truly acid; that is, with  $(\overset{+}{H})$  concentrations greater than 7.0. Formaldehyd was absent in the majority (98.1 per cent.) of urines which were truly alkaline; that is, those possessing  $(\overset{+}{H})$  concentrations less than 7.0. Certain of the tests have been recorded as doubtful. This may be due either to a high dilution of the urines or to an insufficient amount of hexamethylenamin. In general, the intensity of the formaldehyd reaction was directly proportional to the  $(\overset{+}{H})$  concentration of the urine; that is, the higher the acidity the more intense the red color, and the lower the acidity the less intense the color, so that oftentimes only traces of color could be detected. The individual protocols showed that hexamethylenamin was present in all of the fractional specimens voided.

The demonstration of free formaldehyd in the freshly voided urine, and even in the urine from the pelvis of the kidney in the dog experiments, shows that liberation must occur within the kidney, and that the formaldehyd effect must begin somewhere in the tubules. It is, therefore, possible for antiseptic action to occur at least in the pelvis of the kidney.

TABLE 9.—RELATION OF HYDROGEN ION CONCENTRATION TO LIBERATION OF FORMALDEHYD FROM HEXAMETHYLENAMIN IN FRESHLY VOIDED URINES

	Hydrogen Ion Concentration	Total No. of Hexamethylenamin Urines with Formaldehyd			Hydrogen Ion Concentration	Total No. of Hexamethylenamin Urines with Formaldehyd	
		Present	Absent			Present	Absent
↑ Increasing alkalinity  Neutrality—>  ↓ Increasing acidity	7.4 ..	....	4	↓ Increasing acidity	6.15 ..	1	....
	7.3 ..	....	8		6.1 ..	5	....
	7.25 ..	....	2		6.0 ..	6	....
	7.2 ..	....	28		5.95 ..	26	....
	7.16 ..	....	3		5.9 ..	2	....
	7.15 ..	....	2		5.85 ..	6	....
	7.1 ..	1	6		5.82 ..	1	....
	7.0 ..	1	4		5.8 ..	8	....
	6.98 ..	....	1		5.75 ..	5	....
	6.95 ..	2	1		5.7 ..	3	....
	6.92 ..	1	....		5.65 ..	5	....
	6.9 ..	2	....		5.6 ..	4	....
	6.85 ..	2	....		5.58 ..	1	....
	6.7 ..	9	....		5.55 ..	1	....
	6.65 ..	1	....		5.5 ..	3	....
	6.64 ..	2	....		5.47 ..	1	....
	6.6 ..	7	....		5.45 ..	1	....
	6.55 ..	1	....		5.4 ..	4	....
	6.52 ..	1	....		5.35 ..	1	....
	6.5 ..	9	....		5.3 ..	3	....
	6.47 ..	3	....		5.25 ..	1	....
	6.45 ..	4	....		5.2 ..	3	....
	6.38 ..	1	....		5.19 ..	6	....
	6.35 ..	4	....		5.1 ..	10	....
	6.3 ..	8	....		5.0 ..	16	1
	6.25 ..	2	....		4.85 ..	1	....
	6.24 ..	1	....		4.48 ..	1	....
	6.2 ..	9	....		Total .	196	60

#### COMMENT ON TABLE 10

*Standing Urines.*—It will be readily seen from Table 10 that practically identical results were obtained with old urines as with freshly voided specimens. That is, practically all of the urines which were truly acid showed the presence of formaldehyd, while those which were truly alkaline liberated no formaldehyd. It was stated that some of these urines stood longer than twenty-four hours. Among these were urines which were truly alkaline ( $\text{H}^+$ ) concentrations less than 7.0, and after

standing two to three days they still showed no formaldehyd, although the quantity of hexamethylenamin present was abundant as was indicated by the character of the bromin-water precipitate. No formaldehyd, therefore, could be liberated in a bladder containing alkaline urine. It is conceivable, however, that in such cases formaldehyd liberated in the kidney might continue to act for a time in the bladder. This phase needs further investigation.

*Cerebrospinal Fluid.*—Table 8 shows that hexamethylenamin, but no free formaldehyd, appears in the cerebrospinal fluids of dogs. The quantities obtained were usually so small that not enough of the fluid was available for ( $\overset{+}{H}$ ) concentration determinations.

TABLE 10.—RELATION OF HYDROGEN ION CONCENTRATION TO LIBERATION OF FORMALDEHYD FROM HEXAMETHYLENAMIN IN STANDING URINES

	Hydrogen Ion Concentration	Total No. of Hexamethyl-enamin Urines with Formaldehyd			Hydrogen Ion Concentration	Total No. of Hexamethyl-enamin Urines with Formaldehyd	
		Present	Absent			Present	Absent
<div>↑ Increasing alkalinity → Neutrality ← Increasing acidity ↓</div>	7.4 ..	....	1	<div>— ↓ Increasing acidity</div>	6.64 ..	1	....
	7.25 ..	....	1		6.5 ..	1	....
	7.2 ..	....	1		6.47 ..	1	....
	7.16 ..	1 (?)	1		6.45 ..	2	....
	7.0 ..	....	1		6.4 ..	1	....
	6.95 ..	2	....		6.38 ..	1	....
	6.94 ..	....	1		6.37 ..	1	....
	6.9 ..	1	1		6.35 ..	5	....
	6.89 ..	1 (?)	....		6.32 ..	1	....
	6.85 ..	1	....		6.3 ..	4	....
	6.75 ..	2	....		Total .	28	8
	6.72 ..	1	....				
	6.7 ..	1	1 (?)				

Observations as follows were made on patients.<sup>37</sup> Hexamethylenamin in different doses was given by mouth, and after a given interval of time, lumbar puncture was performed and the fluid was collected directly into a test-tube. The phloroglucin test for free formaldehyd was immediately applied directly to the fluid. The same mixture was then distilled after the addition of a little sulphuric acid and the same test was applied to the distillate. When positive this indicated the presence of hexamethylenamin. The data obtained have been placed in Table 11.

37. We wish to express our thanks to Drs. McClelland, Gammon, MacGregor, Lowe and Scott of the medical service of Lakeside hospital for collecting fluids from these patients.

TABLE 11.—EXCRETION OF HEXAMETHYLENAMIN AND FORMALDEHYD IN CEREBROSPINAL FLUID

Patient	Diagnosis	Character of Cerebro-spinal Fluid	For- maldehyhd Present + Absent —	Hexa- methyl- enamin Present + Absent —	Hydrogen Ion Concen- tration	Time of Lumbar Puncture After Administra- tion of Drug, Hrs.	Quantity of Hexa- methyl- enamin Given by Mouth, gr.	Number of Doses	Remarks
H. ....	Leprosy	Clear; colorless	—	—	...	¼	60	1	+ Blood, (H) = 7.2
Mrs. E. ....	Alcoholic neuritis	Clear; colorless	—	+*	7.1	½	60	1	
J. R. ....	Tubes	Clear; colorless	—	+	...	¾	60	1	
A. MeM. ....	Cerebral arterio- sclerosis	Clear; colorless	—	+	...	5/6	60	1	
S. S. ....	Epilepsy	Clear; colorless	—	+	...	1	60	1	
Mrs. S. ....	Cerebro- spinal lues	Clear; colorless	—	+	7.3	1½	60	1	Had received 40 grains of boric acid
C. ....	Cerebro- spinal lues	Clear; colorless	—	+	...	2	40	1	
F. Y. ....	Chronic gastritis	Clear; colorless	—	+	...	3	60	1	
M. V. ....	Pneumo- cocic meningitis	Amber; turbid; pneumococci present	—	+	...	18	180	9	Hexamethyl- enamin given; 20 gr. every two hours
M. V. ....	Pneumo- cocic meningitis	Amber; turbid; pneumococci present	—	+	...	84	840	42	Hexamethyl- enamin given; 20 gr. every two hours (continued)

\* = Trace.



## COMMENT ON TABLE 11

The results as shown in Table 11 indicate that hexamethylenamin appears in the cerebrospinal fluid about thirty to forty-five minutes after administration. This is practically confirmative of Hald,<sup>19</sup> who reported forty-five minutes. It is seen that no free formaldehyd appeared at any time in any of the fluids examined. The dosage varied from 40 grains (3.2 gm.) to 840 grains (54.5 gm.). Patient M. V., who had received 20 grains every two hours for fifty-one hours, whose total dosage amounted to 1,020 grains (66.2 gm.), and whose fluid was obtained on three consecutive days, showed no free formaldehyd at any time. At the end of this time there was marked vesical irritation with hematuria and the urine showed a high concentration of formaldehyd.

The ( $\overset{+}{H}$ ) concentration was not obtainable in all of the fluids on account of their limited supply. Patients S. and E. showed ( $\overset{+}{H}$ ) concentrations of 7.3 and 7.1, respectively. It is known that the ( $\overset{+}{H}$ ) concentration of cerebrospinal fluid is about like that of serum. This is confirmed by the fluids of other patients which have been examined by us; that is, the tendency of the fluid is to be slightly alkaline or neutral. This is, undoubtedly, the reason why free formaldehyd is not liberated from hexamethylenamin in cerebrospinal fluid. The absence of formaldehyd indicates that there can be no antiseptic action in the spinal canal.

*Other Body Fluids.*—Table 8 shows that hexamethylenamin appears in the following fluids: pleural, pericardial, synovial, aqueous and vitreous humors. In no case could free formaldehyd be detected. Usually not enough of the individual fluids could be obtained for determinations of the ( $\overset{+}{H}$ ) concentrations, but an examination of the fluids of two other healthy dogs showed that they are all neutral (7.0).

A child suffering with a tuberculous pleuritis was given one gram of hexamethylenamin before the chest puncture was made. Two hundred c.c. of a nearly colorless, but slightly turbid fluid were obtained, and this showed the presence of hexamethylenamin, but no free formaldehyd. The ( $\overset{+}{H}$ ) concentration was 7.1. In other words, it was slightly alkaline and this explains why no free formaldehyd was liberated.

In April, 1911, the vaginal dressings<sup>38</sup> of four parturient women receiving hexamethylenamin were examined for the presence of free formaldehyd and hexamethylenamin. The lochia of these patients were light-straw colored, having been collected in each case about the tenth day of parturition. Two gm. of hexamethylenamin were given to each

38. We are indebted to Prof. Arthur H. Bill of the Department of Obstetrics for this material.

patient and the vaginal dressings were removed at the end of six to eight hours. These were then extracted with distilled water. The phloroglucin test for formaldehyd was then applied directly to the extracts and a portion was distilled with sulphuric acid. Hexamethylenamin was found present in each case, but no free formaldehyd.

Gastric juice, on account of its relatively high acidity, should liberate formaldehyd readily. Accordingly, we found both free formaldehyd and hexamethylenamin in stomach washings ( $\overset{+}{H} = 4.0$ ) of a patient receiving hexamethylenamin.

#### CONCLUSIONS

Following the administration of hexamethylenamin, the substance itself can be detected in all of the body fluids. Free formaldehyd does not appear in any of the body fluids which are neutral or truly alkaline. This includes blood, cerebrospinal, pleural, pericardial and synovial fluids, aqueous and vitreous humors, saliva, bile and some urines. Urines which are truly acid and contain hexamethylenamin practically always contain formaldehyd. Urine is the only body fluid, except gastric juice, which exhibits true acidity, hence, it is here only that liberation of formaldehyd from hexamethylenamin can take place.

#### VIII. *Effect of the Administration of Monosodium Phosphate on the Reaction of Urine and Liberation of Formaldehyd*

Since the acidity of urine depends on the presence of monosodium phosphate ( $\text{NaH}_2\text{PO}_4$ ) and disodium phosphate ( $\text{Na}_2\text{HPO}_4$ ), it would be interesting to know if the acidity of urine can be increased by the administration of the monophosphate. Its administration has been advised for this purpose. The acidity could conceivably be increased in two ways: 1. By increasing the concentration in the urine through an increased absorption of the phosphate. 2. By diminishing the relative concentration of the bases of the tissues at large, thereby simultaneously increasing the concentration of the monosodium phosphate which then would be excreted and increase its concentration in the urine. This is perhaps more probable because of the limited absorbability of the phosphate, as indicated by the experiment, a statement of which follows:

Subject P. J. H. took 13 gm. of monosodium phosphate at irregular intervals within three hours and forty minutes on a starvation day. The urines were collected in fractions and the acidities of the urines as well as the stools were determined according to the ( $\overset{+}{H}$ ) concentrations. On previous occasions (two or three days preceding the experiment) the urine of P. J. H., while on a constant and mixed diet had been collected, and the ( $\overset{+}{H}$ ) concentrations were determined in the fractional specimens throughout these periods. The urine possessed constantly a low acidity, that is, the average ( $\overset{+}{H}$ ) concentration was about 6.8. Protocol of the phosphate experiment is given in full (Table 12).

During the day previous to the experiment he had received 2 gm. of hexamethylenamin. On the day of the experiment, breakfast consisting of 1 cup of coffee and 2 biscuits with butter was taken at 8 a. m.; no other food was taken during the day; about 200 c.c. of water was taken with each dose of the phosphate.

TABLE 12.—RESULT OF EXPERIMENT WITH MONOSODIUM PHOSPHATE

Time		Hydrogen Ion Concen- tration	Hexa- methyl- enamin	For- maldehyd	Remarks
8:30 a. m.	Passed stool, well-formed, same as usual .....	....	...	...	
10:35	Voided urine .....	6.45 (?)	+	+	
11:00	Took 2 gm. $\text{NaH}_2\text{PO}_4$ ...	....	...	...	
11:10	Voided urine .....	7.2	+	—	
11:20	Voided urine .....	6.7	+*	—	
11:40	Voided urine .....	6.55	—	—	
11:45	Took 2 gm. $\text{NaH}_2\text{PO}_4$ ...	....	...	...	
12:03 p. m.	Voided urine .....	6.35	—	—	
12:05	Took 2 gm. hexamethyl- enamin .....	....	...	...	
12:15	Took 4 gm. $\text{NaH}_2\text{PO}_4$ ...	....	...	...	
12:20	Voided urine .....	6.38	+*	—	
12:40	Voided urine .....	6.38	+†	+	Sensation of warmth in gastric region.
12:43	Took 1 gm. $\text{NaH}_2\text{PO}_4$ ...	....	...	...	
1:05	Voided urine .....	6.30	+†	+†	Watery stool; suggestion of nausea.
1:35	Voided urine .....	5.70	+†	+†	Profuse watery stool; nausea.
1:40	Took 2 gm. $\text{NaH}_2\text{PO}_4$ ...	....	...	...	
2:15	Voided urine .....	5.85	+†	+†	
2:18	Took 1 gm. $\text{NaH}_2\text{PO}_4$ ...	....	...	...	
2:21	Profuse watery stool....	....	...	...	
2:30	Voided urine .....	5.82	+	+	Profuse watery stool, clear almost like water; nausea.
2:40	Took 1 gm. $\text{NaH}_2\text{PO}_4$ ...	....	...	...	
3:08	Took 0.5 gm. hexamethyl- enamin .....	....	...	...	
3:13	Voided urine .....	5.0	+	+	
3:40	Voided urine .....	4.85	+	+	Watery stool; light amber H—; F—; + (H) = 7.0
4:25	Voided urine .....	5.40	+	+†	
4:38	Voided urine .....	5.75	+	+†	Desire to micturate after bladder is emptied.
4:40	Watery stool .....	7.0	+†	—	F, by phloroglucin direct; H, by distillation.
4:48	Voided urine .....	5.80	+†	+†	
4:52	Watery stool .....	7.0	+†	—	Tests same as at 4:40; 5:00 to 7:00 p. m., five watery stools.

\* Trace. † Strong.

All of the stools, from 1:35 p. m. to 4:52 p. m., inclusive, gave marked white flocculent precipitates with magnesia mixture.

## COMMENT ON TABLE 12

It is seen from Table 12 that the acidity of the urine gradually increased during the course of the administration of the phosphate, a result similar to certain ones obtained by L. J. Henderson and Palmer.<sup>39</sup>

In the fore part of the experiment, the  $(\overset{+}{H})$  concentration of the urine was very low, that is, 7.2 and 6.7. At the end of four hours and forty minutes after the administration of the salt was begun, the  $(\overset{+}{H})$  concentration reached its maximum; that is, 4.85. This corresponded practically to the strength of a standard solution consisting of monosodium phosphate alone. In other words, at this time practically pure acid phosphate was being excreted in the urine. After this, the  $(\overset{+}{H})$  concentration gradually began to diminish again, although the urine still remained markedly acid. Throughout the course of the experiment a more marked acidity of the urine was obtained than has been observed for some time in this subject. Two gm. of hexamethylenamin were taken during the course of the experiment. This appeared in the urine in fifteen minutes after administration and formaldehyd was present in all of the fractions which were truly acid. It was noticed that as the acidity increased the intensity of the formaldehyd reaction increased also.

During the experiment several watery stools were passed. The first stool appeared after 9 gm. of the salt had been taken, and within two hours and five minutes after the first dose was administered. In all, twelve stools appeared within six hours after the first stool. All of the stools were very thin and watery, straw-colored and contained a few shreds of mucus and presumably a little bacterial rest. On shaking, they frothed, and possessed an odor suggestive of fresh blood, consisting to some extent, perhaps, of serum. The  $(\overset{+}{H})$  concentrations of each of three of the later specimens was 7.0; that is, the reaction was neutral, and on the addition of magnesia mixture they gave voluminous precipitates showing that they contained a large quantity of phosphate. The stools showed the presence of hexamethylenamin, but did not exhibit free formaldehyd.

This shows that the monosodium phosphate in its passage through the gastro-intestinal tract became neutralized and was to a large extent eliminated unabsorbed. It can be inferred from this that there was a depletion of bases of the blood and tissues at large to such an extent as to neutralize the acid phosphate. Further evidence of this exists in the fact that the acidity of the urine had been markedly increased. That is, the urine contained a high concentration of the acid monosodium phos-

39. Henderson, L. J., and Palmer: Jour. Biol. Chem., 1913, xiv, 81.



phate because of the impoverishment by the basic phosphate of the available sources for urine formation (blood and tissues at large). It is, therefore, possible to increase the true acidity of the urine at the expense of the bases of the blood and tissues at large.

Subject R. J. C. took 5 gm. of monosodium phosphate (1 gm. to a dose at intervals of a half hour) within three hours. One gm. of hexamethylenamin was taken with the first dose of the phosphate. On a previous day the urine had been rendered alkaline by taking sodium bicarbonate. The average <sup>+</sup>(H) concentration of six fractional urines was 7.25 and hexamethylenamin was present in all specimens, but no free formaldehyd. The next three specimens obtained

TABLE 13.—EFFECT OF SODIUM BICARBONATE ON LIBERATION OF FORMALDEHYD

Time		Hydrogen Ion Con- centration	Hexa- methyl- enamin	For- maldehyd
11:00 a. m.	Voided urine .....	6.45	+*	++
11:15	Voided urine .....	6.5	+*	+
11:20	Voided urine .....	6.52	+*	+
11:30	Voided urine .....	5.1?	+*	+
11:33	Took 1 gm. NaHCO <sub>3</sub> ....	....	...	...
11:45	Took 1 gm. NaHCO <sub>3</sub> ....	....	...	...
11:52	Voided urine .....	6.7	+*	++
11:55	Took 1 gm. NaHCO <sub>3</sub> ....	....	...	...
12:05 p. m.	Took 1 gm. NaHCO <sub>3</sub> ....	....	...	...
12:20	Voided urine .....	6.92	+*	++
12:40	Voided urine .....	7.0	+*	++?
12:58	Voided urine .....	7.2	+*	—
1:20	Voided urine .....	7.4	+*	—
1:50	Voided urine .....	7.4	+*	—
5:00	Voided urine .....	7.4	+	—
5:30	Voided urine .....	7.2	+	—

\* Strong. † Trace. + = present. — = absent.

within the next three hours showed <sup>+</sup>(H) concentrations of 6.6, 6.2 and 5.95, respectively. All of the specimens showed the presence of free formaldehyd. This experiment shows that the alkaline urinary fractions contained hexamethylenamin, but no free formaldehyd, and that as soon as the urine became truly acid, free formaldehyd was detectable. Presumably, the change in the reaction of the urine, that is, from alkalinity to acidity, was due to the monosodium phosphate.

CONCLUSIONS

In an individual whose urine commonly shows a low degree of acidity it is possible to increase the acidity; that is, the <sup>+</sup>(H) concentration, by the administration of monosodium phosphate. This gives rise to a profuse diarrhea and the stools contain the phosphate neutralized. Hexamethylenamin given by mouth at the same time appears unchanged in the stools, but formaldehyd is present in all fractions of urine which are

truly acid. The intensity of the formaldehyd reaction appears to be directly proportional to the acidity of the urine.

*IX. The Administration of Alkali Renders the Urine Alkaline and Inhibits the Liberation of Formaldehyd from Hexamethylenamin*

This was shown in the following manner. Sodium bicarbonate was administered by mouth during a course of hexamethylenamin medication. The urine was collected in fractions and the phloroglucin test was applied to the freshly voided urine. The reactions of the fractional urines were determined in the usual way. A protocol illustrative of the effect of sodium bicarbonate is here presented in detail.

Subject P. J. H., took 2 gm. of hexamethylenamin at 10:40 a. m. About 200 c.c. of water was taken at four different times.

For results, see Table 13.

A second experiment on another subject gave similar results.

COMMENT ON TABLE 13

It is seen from Table 13 that the administration of sodium bicarbonate diminishes the  $(\overset{+}{H})$  concentration of the urine (also observed by L. J. Henderson and Palmer<sup>39</sup>), so that the urine becomes truly alkaline. Previous to the administration of the bicarbonate, several fractions of urine showed an average  $(\overset{+}{H})$  concentration of 6.5. This was then reduced to a concentration of 7.4, which indicates true alkalinity. Hexamethylenamin and formaldehyd were present in all of the fractions which showed true acidity, but formaldehyd failed to appear when the urine became truly alkaline  $(\overset{+}{H})$  concentrations of 7.2 and 7.4.

Six dispensary patients were given variable doses of potassium citrate together with hexamethylenamin. From some the urines were collected while the patients remained at the dispensary; others took the citrate over night and urines were collected the following day. In all, thirty-four fractional urines were collected, and the  $(\overset{+}{H})$  concentration readings in the different patients ranged as follows: Patient V., 6.9 to 7.15; Patient J., 7.16 to 7.3; Patient K., 6.95 to 7.2; Patient F., 7.0 to 7.4; Patient P., 6.95 to 7.3; Patient D., 7.16 to 7.3. Hexamethylenamin was present in all of the freshly voided specimens, but no formaldehyd could be detected. The specimens were allowed to stand twenty-four hours and the phloroglucin test was again applied. In none of the specimens could any free formaldehyd be detected.

Disodium phosphate ( $Na_2HPO_4$ ) and hexamethylenamin were administered to one subject on two different occasions; once, to another subject. Three gm. of the salt were taken each time and fractional

specimens of urine were collected as in previous experiments. The urines at all times were truly acid and contained hexamethylenamin and free formaldehyd.

#### CONCLUSIONS

The administration of sodium bicarbonate and potassium citrate alters the reaction of acid urine to true alkalinity, and the liberation of formaldehyd from such urines containing hexamethylenamin is inhibited.

#### *X. The Rationale of Hexamethylenamin Therapy*

From the foregoing correlation of chemical experiments with bacteriological and clinical data, it is clear that hexamethylenamin acts as an antiseptic only in proportion as it liberates formaldehyd, and that this liberation can only occur when the reaction is truly acid. Free formaldehyd is an efficient bactericide in comparatively high dilutions. Such an efficient concentration, no doubt, can be attained in normally acid urine. It is only in vesico-urinary conditions where we can expect any beneficial therapeutic action after the administration of hexamethylenamin, because urine is the only body fluid whose reaction may be altered, if necessary, to such an extent that liberation of formaldehyd from hexamethylenamin will be facilitated. This is not possible with other body fluids inasmuch as their neutrality or a slight tendency to alkalinity is rigidly maintained. No beneficial therapeutic responses, therefore, are to be expected in meningeal infections, infections of the cerebrospinal fluid or about the spinal cord, or of the infections of the ear, eye, synovial, pericardial or pleural fluids. We have not yet determined the position of bile in this respect. On account of the rapid absorption of hexamethylenamin and of the usual alkalinity of the bowel contents, no bactericidal action can be expected in the intestinal canal. It was shown in our experiments that the administration of monosodium phosphate ( $\text{NaH}_2\text{PO}_4$ ) does not alter the reaction of the fluid from the intestines, and that the hexamethylenamin present under such conditions passes through the bowel unchanged. The administration of the monosodium phosphate increases the true acidity of the urine and the concentration of formaldehyd in such a urine containing hexamethylenamin is markedly increased. In the urine, therefore, an opportunity presents itself to increase the efficiency of hexamethylenamin therapy. This can be accomplished by the administration of the monosodium phosphate until the urine when tested with the phloroglucin reagent gives a reaction for formaldehyd. This test indicates quite closely the turning point in the reaction of urine. That is, at approximately the point of neutrality or beginning alkalinity it will no longer give a positive formaldehyd test with a hexamethylenamin urine, but as soon as such a urine begins to be truly acid it gives a positive test for formaldehyd. A urine which has been previously alka-

line, that is, possessing a hydrogen ion concentration of 7.2, can be rendered acid; that is, increased to a hydrogen concentration of 4.85 within approximately five hours after the administration of 13 gm. (200 grains) of the phosphate. If the occurrence of diarrhea is objectionable, the dosage of the phosphate may be reduced.

On the other hand, the administration of alkalies, such as sodium bicarbonate ( $\text{NaHCO}_3$ ) or potassium citrate, entirely prevents the benefits of hexamethylenamin, and it is, therefore, irrational to prescribe them together. Our data were obtained largely from normal individuals, but so far as urine is concerned it would perhaps make little or no difference as to the behavior of hexamethylenamin in urines in various pathological conditions, since it is the reaction which is the essential factor in determining the decomposition. Recently, it has been shown by Henderson and Palmer<sup>40</sup> that urines in a great variety of pathological conditions are truly acid; that the tendency in many conditions is to a higher acidity than is normally found in urine of the average person. Such pathological urines would facilitate the liberation of formaldehyd from hexamethylenamin.

Concerning other body fluids our pathological data are as yet too scant to offer any suggestions in this direction. We have had thus far ten cerebrospinal fluids and one pleural exudate, and from the fact that they contained no free formaldehyd, it may be contended that no bactericidal action could have been expected. It is possible, if not probable, that the hydrogen ion concentration in certain other pathological fluids may be high enough to facilitate the liberation of formaldehyd from hexamethylenamin. With this end in view, the study of various pathological fluids is being continued.

We wish to express our thanks to Professor Sollmann for advice, and for criticism of the manuscript; to Professors Hoover and Phillips, and to Dr. Lester H. Taylor of the Medical Service for permission to use clinical material.

#### SUMMARY \*

1. The phloroglucin test is the most delicate and most useful test for free formaldehyd.

2. Alkalies prevent, while acids facilitate the liberation of formaldehyd from hexamethylenamin in all body fluids.

3. The liberation of formaldehyd from hexamethylenamin in pathological fluids obeys the same laws as in other solutions; that is, it can only occur in acid reaction. Even when 0.5 per cent. hexamethylenamin was added to them, not enough formaldehyd was liberated to be bactericidal.

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40. Henderson, L. J., and Palmer: *Jour. Biol. Chem.*, 1913. xiii. 393.

\* The numbers refer to the sections in the text.



4. Hexamethylenamin itself is not bactericidal.
5. Liberation of formaldehyd from hexamethylenamin depends on the excess hydrogen ion concentration of the solution above the neutral point.
6. Previous investigations leave us in doubt as to the behavior of hexamethylenamin in the body.
7. After administration, hexamethylenamin is present, but does not liberate free formaldehyd in blood, cerebrospinal, pleural, pericardial and synovial fluids, vitreous and aqueous humors, and urine when truly alkaline. Formaldehyd is liberated in urine which is truly acid, and in the acid gastric contents.
8. Administration of monosodium phosphate with hexamethylenamin renders the urine acid and facilitates the liberation of formaldehyd.
9. The administration of alkali with hexamethylenamin renders the urine alkaline and inhibits the liberation of formaldehyd.
10. The beneficial therapeutic effects of hexamethylenamin depend on the liberated formaldehyd. Such effects are to be expected principally, if not always, in acid urine only. It is irrational to prescribe alkalies (bicarbonate and citrate) together with hexamethylenamin.

# The Archives of Internal Medicine

Vol. XII

DECEMBER, 1913

No.

## THE "TYPHOID-CARRIER" STATE IN RABBITS AS A METHOD OF DETERMINING THE COMPARATIVE IMMUNIZING VALUE OF PREPARATIONS OF THE TYPHOID BACILLUS

### *STUDIES IN TYPHOID IMMUNIZATION. I\**

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#### INTRODUCTORY

Prophylactic immunization against typhoid fever offers sufficient security against subsequent infection to render its general application advocable. Such application is not only becoming wide-spread and mandatory in segregated, controllable bodies like armies, but has proved very advantageous in communities in general. In surveying the already extensive statistical literature, one becomes convinced of the relative protection against typhoid fever that is enjoyed by those who have been immunized with preparations of the typhoid bacillus; one is likewise convinced that the results obtained and the methods of immunization followed leave much to be desired.

Why should the results of immunization against typhoid infection be less perfect than those obtained in small-pox and rabies? Why should artificial immunization with the typhoid bacillus offer so much less perfect protection than recovery from typhoid fever? In respect to the method employed we may well question why 500,000,000 killed typhoid bacilli is the recommended dose, and why the interval at which this dose should be repeated is arbitrarily set at seven or ten days. The fact is that we possess only too little information of an exact sort about methods of prophylactic immunizing with bacteria in general. In the case of antityphoid immunization, empiricism rather than experimentation, has largely determined the method employed. The results attained are so good that they should be better, and it would seem that the time has arrived to return to animal experimentation in order to save time in determining the ultimately best method of protecting man.

The best evidence that the most satisfactory method has yet to be determined lies in the fact that a number of methods are still being

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\* Submitted for publication Sept. 24, 1913.

advocated. Friedberger,<sup>1</sup> in his exhaustive article on antityphoid immunization, mentions no less than twelve well-recognized preparations of the typhoid bacillus which may be employed. Paladino Blandini<sup>2</sup> has actually endeavored to test the comparative immunizing value of seventeen different typhoid preparations. In addition to the actual preparation to be employed, we have to consider the dose used, the method of estimating the dose, the intervals between doses and the like.

The chief reason why animal experimentation has not done more to perfect the method of typhoid vaccination is the reasonable skepticism which obtains at transferring results attained in a vastly different and artificial infection in animals to a very characteristic human disease. There seems little or no resemblance between the more or less common type of peritonitis produced in the guinea-pig by the typhoid bacillus and actual typhoid fever. Until very recently it has been supposed that nothing resembling typhoid fever in man could be produced in animals. In 1911, Metchnikoff and Besredka<sup>3</sup> described a disease in chimpanzees which had been given food contaminated with the excreta from typhoid fever patients, which resembled in practically all details the human syndrome. They further attempted to utilize this experimental disease as a means of comparing the immunizing value of various typhoid preparations. Their results even in the limited aspect of the problem they attacked can hardly be regarded as conclusive in view of the necessarily small number of animals employed and the fact that their tentative results differ from what is known to be true in man. We shall consider their report more fully later, but here it may be noted simply that desirable as tests might be in the experimental disease most closely simulating typhoid fever in man, it would be impossible from the item of expense and supply alone to carry out any extended experiments on these animals.

#### PRODUCTION OF AN ARTIFICIAL TYPHOID-CARRIER STATE IN RABBITS

It has been known since the observations of Blackstein<sup>4</sup> and Welch<sup>5</sup> in 1891, that living typhoid bacilli injected into the circulation of rabbits can subsequently be recovered in pure culture therefrom, for considerable periods of time. Further work has served to explain the nature of this

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1. Friedberger: *Die Methoden der Schutzimpfung gegen Typhus*, Kraus and Levaditi's *Handbuch der Immunitätsforschung*, 1908, i, 723.

2. Paladino, Blandini: *Profilassi specifica del tifo addominale*, *Ann. d'ig. sper.*, xv, 1905, 295.

3. Metchnikoff and Besredka: *Recherches sur la fièvre typhoïde expérimentelle*, *Ann. de l'Inst. Pasteur*, 1911, xxv, 193.

4. Blackstein: *Intravenous Inoculation of Rabbits with Bacillus Coli Communis*, and the *Bacillus Typhi Abdominalis*, *Bull. Johns Hopkins Hosp.*, 1891, ii, 96.

5. Welch: *Additional Note Concerning the Intravenous Inoculation of the Bacillus Typhi Abdominalis*, *Bull. Johns Hopkins Hosp.*, 1891, ii, p. 121.

"carrier" condition more fully. The injections must be intravenous to yield positive results,<sup>6</sup> and it is evident from the varying success of different observers that not all strains of the typhoid bacillus give equally good results. It is clear from the results of Morgan,<sup>7</sup> Koch,<sup>8</sup> Doerr,<sup>9</sup> and Johnston,<sup>10</sup> that the permanent reservoir of the organism is the gall-bladder, which is invaded within two hours after intravenous inoculation.<sup>6</sup> It may be that, under usual conditions, the circulation is only invaded periodically, which would explain why Johnston failed to get positive cultures from the blood with regularity before the period from the seventh to the tenth day, and also would indicate the reason why Doerr failed to obtain positive blood-cultures after the fourth day. It is certain that failure to produce a carrier should not be judged from a single blood-culture. At all events, cultures from the gall-bladder are positive more constantly and for longer periods than cultures from the blood. Constant lesions of the gall-bladder have been described by Chirolanza in particular and will be further detailed in connection with our own protocols.

The persistence of the typhoid bacillus in the rabbit has been noted for varying lengths of time: in the blood for 58 days (Chirolanza); 109 days (Blackstein); 127 days (Johnston); and in the gall-bladder for even longer periods: 180 days (Uhlenhuth and Messerschmidt<sup>11</sup>); 75 days (Morgan); 120 days (Doerr). The organisms are apparently eliminated through the intestine in which they have been found by Doerr in the mucosa up to 120 days after inoculation. They were likewise repeatedly found in the feces by Morgan and by Johnston.

The actual pathogenicity for rabbits of strains of the typhoid bacillus capable of producing the carrier state in these animals does not appear to have received particular attention from previous observers. Morgan mentions, without further particulars, that two out of twelve of his animals died in twelve hours. Similar observations were made by Chirolanza. Johnston mentions the obtaining of positive cultures from bile and blood in nearly all fatal cases, but does not give the percentage of carrier rabbits that died. Another point of particular interest in view of our employment of the carrier state as a means of demonstrating the

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6. Chirolanza: Experimentelle Untersuchungen über die Beziehung der Typhusbacillen zu der Gallenblase und den Gallenwegen, *Ztschr. f. Hyg. u. Infektionskrankh.*, 1909, lxii, 11.

7. Morgan: Attempts to Produce the Typhoid-Carrier State in the Rabbit, *Jour. Hyg.*, 1911, xi, 202.

8. Koch. Typhusbacillen und Gallenblasen, *Ztschr. f. Hyg.*, 1909, lxii, 1.

9. Doerr: Experimentelle Untersuchungen über das Fortwuchern von Typhusbacillen in der Gallenblase, *Centralbl. f. Bakteriol.*, 1905, Orig. xxxix, 624.

10. Johnston: Experimental Typhoid-Carrier State in the Rabbit, *Jour. Med. Research*, 1912, xxvii, 177.

11. Uhlenhuth and Messerschmidt: Versuche Kaninchen zu Typhusbacillen-Trägern zu machen und sie therapeutisch zu beeinflussen, *Deutsch. med. Wchnschr.*, 1912, xxxviii, 2393.



absence or presence of an active immunity is the uniform presence of agglutinins in positive rabbits (Morgan, Johnston, Doerr).

The typhoid-carrier state in rabbits has been used as a means of testing certain therapeutic measures. Conradi<sup>12</sup> has shown that daily doses of chloroform in oil or milk given by the rectum suffice to cure the carrier rabbits. Hailer and Rimpau<sup>13</sup> have obtained similar results with methyl iodid and iodoform. Johnston has been able to cure the bacteremia by two doses of 20,000,000 killed typhoid bacilli.

To meet our experimental needs, it was necessary that we should be able to produce a typhoid bacillemia with regularity, since the efficacy of a given method of vaccination could be judged only by the failure of properly protected animals to become carriers, whereas the normal controls should give positive results uniformly. The irregularity in the results of other observers was not very encouraging, and our own first results were even less so.

It seems to us that no wholly satisfactory method of obtaining an exact dosage of living bacteria has been devised. Perhaps the least objectionable method consists in employing a given portion of a suspension of a standard agar surface, particularly when the dose is of necessity large, as in the present case. In order to obtain comparable results in different experiments, we have used thick-walled culture-tubes of uniform size (inside diameter 18 mm.), containing 10 c.c. each of a 2 per cent. peptone agar, or in the majority of our experiments agar containing 10 per cent. defibrinated rabbit blood. These tubes are slanted at a fixed angle of about 6 degrees, so that the entire solidified surface is, within a small margin of error, uniform. Twenty-four-hour growths of such cultures suspended in salt solution have been used in amounts representing fractions of an original culture. By plating out these blood-cultures, we estimate that each one contains 1,400,000 million organisms. The blood-cultures are more abundant than corresponding agar-cultures.

The first five strains of *Bacillus typhosus* tested, which included three from the collection of the California State Board of Health Laboratory, Army and Navy Strain 5 obtained from the Cutter Laboratory, and one strain (65), obtained from Professor Zinsser, in an intravenous dose of the entire standard agar-culture previously referred to, failed to produce carriers, to kill, or seriously to affect rabbits which weighed from 1,500 to 2,000 gm. Two other cultures gave positive results, Culture 6 killing a small rabbit in twenty hours, and Strain 3, which had been isolated two weeks previously from a human blood-culture, produced a carrier that gave a positive culture ten days later. The reisolations from these two

12. Conradi: Ueber sterilisierende Wirkung des Chloroform im Tierkörper, Ztschr. f. Immunitätsforsch., 1910, vii, 158.

13. Hailer and Rimpau: Versuche über Abtötung von Typhusbacillen in Organismus, Arbeit. a.d.k. Gsndhtsamte, 1911, xxvi, 409.

animals were made on rabbit-blood agar. All of our subsequent experimental work has been carried on with cultures grown on this medium. To this procedure we attribute, at least in part, our present success in producing a bacteriemia regularly. Since our first success in producing a carrier with a whole standard agar-culture of Strain 3, we have failed with subsequent generations of the culture grown on agar to produce carriers, whereas the blood-agar cultures produce carriers regularly. Inasmuch as the growth on the blood-medium is more abundant, a little less than twice the amount of the agar growth by weight, we have used a whole agar-culture in comparing its infectiousness with half a blood-agar culture. Culture 6, which in a dose of a whole agar-culture killed the first rabbit in twenty hours, in the first blood generation from this animal (Culture 6a) failed to produce a bacteriemia in a dose of one-half of a culture, and the strain was abandoned for Culture 3a and subsequent generations, which gave positive results in this dosage.

A survey of our protocols in which groups of vaccinated rabbits were tested with normal controls, shows that one-half of a standard blood-culture of our Strain 3 of *B. typhosus* produces with almost perfect regularity characteristic pathogenic effects which do not occur in properly protected animals. Smaller doses, as one-quarter of a culture, produce less consistent results. Thus of seventeen control animals used in our latest experiments we find: 1. Three died in from two to twenty-four hours with symptoms of acute intoxication. 2. Thirteen gave one or more positive cultures of *B. typhosus* from the blood<sup>14</sup> at periods from the fifth to the twenty-eighth day, and of these animals five died between the fifth and twentieth day. Of the other eight, only one certainly recovered, as shown by negative cultures from bile and blood; through error, four animals were not observed over a sufficient period of time to know whether they would have died or recovered. 3. Only one animal gave apparently negative results. Cultures from the blood were negative on the sixth, eighth and twentieth day, and when the animal was killed by bleeding on the thirty-fifth day, cultures from the gall-bladder and blood were also negative. The animal, however, showed extreme loss of weight (2,440 to 1,710 gm. on the thirtieth day), and the blood-serum on the thirty-fifth day agglutinated the typhoid bacillus in a dilution of 1 to 2,000.

The carrier state in rabbits is not accompanied by any distinctive or continuous rise in temperature.

Chirolanza has given a full description of the lesions he found associated with the typhoid-carrier state in rabbits, and a few observations

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14. The routine cultures are taken by allowing 10 drops of blood to drop from the carefully cleaned ear vein into bile bouillon. If contaminations are evident, which is rarely the case, the culture is plated out. (Culturally probable typhoid bacilli are tested with an agglutinating serum.)

have also been made by Morgan. The lesions affect the gall-bladder primarily and to a less extent the liver and bile ducts. According to Chirolanza, animals dying in the first few hours after inoculation show cloudy swelling, subcapsular hemorrhages and zonal necroses of the liver. The gall-bladder is not at first affected, but after a day or two the mucosa becomes necrotic. The later changes in this organ are more characteristic and consist in proliferation of the mucosa of the gall-bladder, more rarely a loss of mucosa and thickening of the fibromuscular coat; thickening of the bile-duct and gradual lymphoid infiltration and cirrhosis of the liver.

Although we do not pretend to offer any systematic study of the morbid anatomy of this condition, certain constant lesions are noted in our protocols.

In rabbits in which death is rapid, that is, within twenty-four hours, the gall-bladder shows no changes; the liver shows chronic passive congestion and usually central necrosis of the lobule. We have not studied the intermediate period in any detail, but beginning with the second week certain characteristic changes are to be noted. At necropsy the gall-bladder is uniformly distended and frequently is four or five times the usual size. The bile is usually cloudy or contains visible flocculi and at times a mass of inspissated bile salts is present that fills and distends the gall-bladder. In our experience complete destruction of the mucosa and reduction of the wall to a thickened fibromuscular coat is more frequent than the proliferation described by Chirolanza. We have been unable to convince ourselves of any constant changes in the liver beyond the early zonal necrosis, that is characteristic of bacterial infections in general.

It is of importance in justifying our use of the typhoid-carrier rabbit and in showing that conditions in these animals are analogous to the conditions in typhoid infection in man, to note that the lesions of the gall-bladder in typhoid cases in man are quite similar to those in the infected rabbits. J. Koch has compared these conditions very carefully, using his own and Chirolanza's material. He has further shown that the infection of the bile in man and in the rabbit is through the circulation and not from the intestine by way of the bile duct. The analogy of the lesions in man and in the rabbit is also emphasized by Uhlenhuth and Messerschmidt.<sup>11</sup>

We have further found marked injection of the cecum in two instances, and actual hemorrhage in one instance in carriers, similar to those produced regularly by the toxin of the typhoid bacillus.<sup>15</sup>

We find, then, that the intravenous injection of a uniform amount of a rabbit-blood agar-culture of our strain of *B. typhosus* produces an almost invariable and characteristic syndrome in normal rabbits (94 per

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15. Arima: Ueber die Typhustoxine und ihre pathogene Wirkung, Centralbl. f. Bakteriol., Orig., 1912, lxi, 424.

cent. positive). They either die with acute symptoms of intoxication within the first few hours and without characteristic lesions (18 per cent.) or become more or less chronic carriers (76 per cent.), yielding positive cultures from the blood for two or three weeks, within which time many of them die. Cultures from the gall-bladder of these animals are even more uniformly positive than the blood-cultures, and the bladder itself shows characteristic lesions in the distention and necrosis of the mucosa. The bile becomes inspissated in the majority of cases. Inadequately immunized animals give the same result.

We do not wish at this time to consider in detail the methods of comparative prophylactic immunization with the preparations of the typhoid bacillus that we have employed, but reserve it for a separate report, when our already considerable data have been further amplified. It may suffice to say that we have been comparing dead and living cultures of the organisms, sensitized (Besredka) and unsensitized cultures, and, finally, various fractions of dried and ground bacilli. It will suffice at this point to state that animals which have been fully immunized with, let us say, whole dried and ground preparations of *B. typhosus*, show no ill effects on injection of the standard amount of living blood-culture. The properly protected animals, following the test intravenous inoculation, show no considerable loss of weight, give uniformly negative cultures from twenty-four hours on, and when killed at considerable periods (from twenty-one to thirty-five days) subsequently, present uniformly negative findings at necropsy. In other words, this intravenous method of inoculation has given a nearly perfect method of comparing the relative immunizing property of various preparations of the typhoid bacillus. The small margin of error (6 per cent. in our series) incident to the failure of the exceptional normal animal to give positive results may be corrected by using a sufficient number of animals in testing each immunizing preparation.

It remains to discuss the extent to which we believe we are justified in regarding the carrier condition in rabbits as analogous to a typhoid infection in human beings. The work of recent years on typhoid fever in man has led to considerable readjustment of our ideas as to its pathogenesis. To quote Kolle and Hetsch,<sup>16</sup> we no longer regard typhoid fever as an intestinal disease, but primarily as a bacteriemia. This statement is based first on the fact that blood-cultures taken in the first week of the disease in an appropriate manner yield positive results in 100 per cent. of cases (Brion and Kayser).<sup>17</sup> The bile also gives positive cultures in

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16. Kolle and Hetsch: Die experimentelle Bakteriologie und die Infektions-Krankheiten, Berlin, 1911, i, 250; 3d edit., Urban und Schwarzenberg.

17. Brion and Kayser: Neuere Klinisch-bakteriologische Erfahrungen bei Typhus und Paratyphus, Deutsch. Arch. f. Klin. Med., 1906, lxxxv, 552.



practically all cases and at all stages of the disease (Forster and Kayser<sup>18</sup>). The occurrence of cases of typhoid fever without intestinal lesions is another reason for regarding these typical lesions as secondary rather than primary.<sup>19</sup> There is evidence, indeed, that the lesions in the ileum may be eliminative and secondary to the bacteriemia rather than a primary focus of the disease. The proliferation of lymphoid tissue in the intestine and elsewhere characteristic of the disease has been logically attributed to the typhoid toxins (Mallory) rather than to simple multiplication of the bacillus.

The mere fact, then, that the injection of living typhoid bacilli in rabbits does not lead to the characteristic swelling and necrosis of the agminated follicles of the intestine, as is usual in typhoid fever, is no reason for regarding the rabbit syndrome that we have described as essentially dissimilar from the condition in man. In both rabbit and man the typhoid bacillus remains for considerable periods in the circulating blood, and in both it promptly invades the bile by means of the circulation and not by the bile duct (J. Koch, Doerr, Chirolanza). It remains in both human and rabbit carriers in the gall-bladder even after it has disappeared from the circulation and in both cases it is subsequently eliminated through the intestinal mucosa. The characteristic result of gall-stone formation which may follow typhoid infection in man has also been produced in rabbits by Richardson<sup>19</sup> by giving calcium. It has been noted that the intestinal lesions may more properly be attributed to eliminated toxins of the typhoid bacillus, and it is interesting to note that similar lesions may be produced in rabbits by the use of typhoid toxins<sup>15</sup> instead of living cultures. We have obtained similar results with killed cultures of *B. typhosus* in sufficient doses. Arima produces by means of intravenous injection of his "endotoxin" of *B. typhosus*, bloody diarrhea, hyperemia, swelling and hemorrhage of the intestinal lymph-nodes.

We regard these facts as indicating the very close analogy of infections produced by the typhoid bacillus in man and in the rabbit. Apart from its intrinsic value as a clean-cut experimental method, the rabbit "carrier" condition would seem to offer, next to experimentation on anthropoid apes, the best means for the comparative testing of various immunizing preparations of the typhoid bacillus, designed to be used on man.

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18. Forster and Kayser: Ueber das Vorkommen von Typhusbacillen in der Galle von Typhuskranken und "Bacillenträgern," München. med. Wchnschr., 1905, lii, 1473.

19. Possett: Atypische Typhusinfektion. Typhus ohne Darmerkrankung. Lubarsch and Ostertag: Ergebn. d. allg. Pathol., 1912, xvi, 184.

19. Richardson: On the Role of Bacteria in Gall-Stones, Jour. Boston Soc. Med. Sc., 1899, iii, 79.

# AGGLUTINABILITY OF BLOOD AND AGAR STRAINS OF THE TYPHOID BACILLUS

## STUDIES IN TYPHOID IMMUNIZATION. II\*

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The growth of the typhoid bacillus on an agar-medium containing 10 per cent. defibrinated rabbit blood, which we have found of advantage in our experiments, differs in certain respects from the growth on plain agar. We have already noted that the blood-agar cultures are more abundant. A concrete example of the amount of growth produced on each medium may be drawn from certain of our vaccine preparations to be more fully described later. To produce growths of *Bacillus typhosus* for immunizing vaccines we inoculate one-quart Blake bottles containing 100 c.c. of medium with a few cubic centimeters of a twenty-four-hour bouillon-culture of the organism. In one experiment nine Blake bottles of 2 per cent. agar were inoculated with a plain bouillon-culture of *B. typhosus* No. 3; at the same time, nine Blake bottles of 10 per cent. blood-agar were inoculated with a blood-bouillon culture of *B. typhosus* No. 3b. After incubation at 37° C. (98.6° F.) for two days, the collected agar and blood-agar growths were suspended each in 220 c.c. of normal saline, filtered through cotton and precipitated with 300 c.c. of absolute alcohol. After centrifugalization, the separate precipitates were again centrifugalized and dried over sulphuric acid for two days. These dried cultures were ground and weighed with the following results: Blood-growth, 0.675 gm.; agar-growth, 0.36 gm. It will thus be seen that the growth of the organism is almost twice as abundant on 10 per cent. blood-agar as on plain agar. Not only is the growth of the blood organism more abundant, but its morphology differs distinctly from the agar-strain. When examined in hanging-drop preparations, the blood-strain of the typhoid bacillus is larger, thicker and tends to grow in chains. It remains, however, quite as motile as the agar variety.

Associated with the morphological change that the organism undergoes when grown on rabbit blood, and we believe in some way dependent on it, are certain distinct and important variations in the agglutinability of these organisms by antityphoid serum. The ultimate identification of bacilli isolated from the blood-stream of suspected typhoid-carrier rabbits

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depends on their agglutination by means of a potent antityphoid serum. Our first attempt to agglutinate such a culture (*B. typhosus* 3a), which culturally and morphologically agreed with the characteristics of *B. typhosus*, with a potent antityphoid serum from the rabbit, was a complete failure. The rabbit furnishing this serum had been immunized by repeated inoculations of killed agar-cultures of *B. typhosus* suspended in saline and its serum agglutinated the stock cultures with which we were working in dilutions of from 1:10,000 to 1:20,000. The failure of this potent diagnostic serum to agglutinate the cultures derived from the suspected carrier in dilutions of 1:100, in view of the fact that these cultures were isolated on a blood-medium, whereas they had been growing on an agar-medium before inoculation into the rabbit, led us to suspect that we might be dealing with the phenomenon described by Bordet and Sleswijk with the whooping-cough bacillus.

Bordet and Sleswijk,<sup>1</sup> it will be remembered, found that the serum of an animal that had been immunized by injections of agar-growths of the Bordet-Gengou whooping-cough bacillus would clump similar agar-strains, but had no effect on blood-culture growths of the same organism. The serum of an animal immunized by means of the blood organisms would, however, agglutinate both varieties of whooping-cough bacilli. With these observations in mind we immunized several rabbits with blood-cultures of *B. typhosus*, and compared the agglutinating properties of their serums with the serums of rabbits that had received agar-cultures of the organism.

In Table 1 are grouped the results from a series of experiments with antisera from rabbits obtained in the manner indicated. The first three serums in the vertical columns are from rabbits that had been given injections of agar-cultures of *B. typhosus*; the other six serums are from animals that received blood-cultures (*B. typhosus* 3a).

Table 1 shows clearly the following facts: An antityphoid serum from a rabbit that has been immunized by blood-agar cultures of *B. typhosus* has the property of agglutinating indiscriminately, to the limit of its potency, both blood and agar-cultures of the organism.<sup>2</sup> The serum of a rabbit immunized against agar-cultures agglutinates the agar organism only. The inagglutinability of blood-cultures by antiagar serum is not due to some property acquired in the living animal, as similar results can be obtained by simple subculture for two generations of the agar-strain on blood-medium. The agglutinability may be restored by retransference to the agar-medium. Growth in bile produces the same results as growth in blood.

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1. Bordet and Sleswijk: Séro diagnostic et variabilité des microbes suivant le milieu de culture. Ann. de l'Inst. Pasteur, 1910. xxiv. 476.

2. It may be noted, however, that the agglutination of the blood-strains invariably proceeds more slowly than that of agar-cultures.

The variation in the morphological appearance of the blood-agar cultures suggests that the failure of these organisms to agglutinate might be due to some physical difference acquired by growth on this medium. It is known that the agglutinability of members of the *mucosus capsulatus* group of bacteria, for instance, is dependent on the absence of a capsule.<sup>3</sup>

Two recent articles seem to prove that the typhoid bacillus may be capsulated either under exceptional conditions, or perhaps as a rule, provided proper strains are used. Kuhnemann<sup>4</sup> found capsules could be

TABLE 1.—LIMITS OF AGGLUTINATION OF AGAR AND BLOOD-AGAR STRAINS OF *B. TYPHOSUS* WITH SERUMS DERIVED FROM RABBITS BY IMMUNIZATION WITH AGAR OR WITH BLOOD-AGAR CULTURES

Strains of <i>B. Typhosus</i>	Antiagar Culture Rabbits			Antiblood Culture Rabbits					
	4	5	6	19	39	42	50	51	52
Agar 5 .....	20,000	10,000	2,000	2,000	4,000	2,000	2,000	500	8,000
Agar 3 .....	10,000	.....	.....	.....	.....	.....	.....	1,000	1,000
Blood 3a .....	100*	100*	100*	2,000	.....	2,000	1,000	1,000	500
Blood 3b .....	100*	.....	.....	.....	1,000	.....	.....	.....	1,000
Blood 3 <sup>2</sup> .....	100*	.....	.....	.....	1,000	.....	.....	.....	.....
Blood 3 <sup>3</sup> .....	100*	.....	.....	.....	.....	.....	.....	.....	1,000
Blood 5 <sup>3</sup> .....	100*	.....	.....	.....	.....	.....	.....	.....	2,000
Bile 3 .....	100*	.....	.....	.....	1,000	.....	.....	.....	.....
Bile 5 .....	100*	.....	.....	.....	1,000	.....	.....	.....	.....

\* Negative.

The figures indicate the highest dilutions of the serum at which a positive agglutination was obtained. The macroscopic method was uniformly employed, each tube containing a dilution of the serum in 1 c.c. of salt solution plus a uniform amount of the organism; in the case of the suspended cultures from blood or agar, this was obtained by standardizing the cultures to a uniform turbidity and using 0.1 c.c. The final result was read after the tubes had stood for two hours at room temperature and then over night in the ice-chamber. No doubtful or partial results are noted and the results were controlled by adding each suspension to salt solution (1 c.c.). The "bouillon-bile" cultures consisted in adding 1 c.c. of a forty-eight-hour culture on this medium to the serum dilution. In the left hand column are the cultures of *B. typhosus* tested in relation to their origin and the mediums on which they had been grown before being tested. "Agar 3" and "Agar 5" refer to the original strains employed. "Blood 3a" and "3b" indicate the Culture 3 passed through one or two carrier rabbits respectively; "Blood 3<sup>2</sup>," "3<sup>3</sup>," "5" indicates that the respective cultures had been simply transplanted from agar on the blood-medium for two or three generations with twenty-four hours between transplants. The bile cultures of five and three were subcultured twice on bile-bouillon before being tested.

3. Beham: Die agglutinatorischen Eigenschaften der Kapselbazillen, etc., Centralb. f. Bakteriologie, 1912, Abt. I, Orig., lvi, 110; Fitzgerald: Agglutination of Encapsulated Bacteria, Proc. Soc. Exper. Biol. and Med., 1912, x, 52.

4. Kuhnemann: Ueber Kapselbildung beim Typhusbazillen, Centralbl. f. Bakteriologie, 1911, lvii, 497; Zur Identifizierung des Bacillus Faecalis Alcaligenes, Centralbl. f. Bakteriologie, 1911, lvii, 469.



demonstrated by his modification of Loeffler's flagellum stains on typhoid bacilli that had been treated and clumped by fresh serum of young rabbits. He is inclined to regard this capsule formation as a protective mechanism on the part of the typhoid bacillus, as he found it only in such specially treated cultures. Carpano,<sup>5</sup> on the other hand, regards the failure hitherto to stain capsules on the typhoid bacillus as a purely technical matter, and he has succeeded in staining them on strains of the micro-organism on various culture-mediums.

Our own experiments with these stains have been convincing.<sup>6</sup> We had thought it might be possible to show capsules on the blood organisms with one of these stains while the corresponding agar-culture remained free, thereby indicating the correspondingly greater resistance to agglutination that is shown by the blood-strains. Such results, however, were not realized. With Kuhnemann's method the clear-cut capsules were found about all typhoid bacilli treated as he suggests with fresh young rabbit serum. We differ with him, however, in finding a considerable number of capsulated bacilli in twenty-four-hour preparations of both agar and blood-agar cultures. With Carpano's method we had no difficulty in demonstrating capsules on both the agar and blood-agar cultures. They are found surrounding each organism, clearly cut and uniformly. We have not succeeded, then, in demonstrating any structural basis for the differences in agglutinability of the agar and blood-agar strains of the typhoid bacillus.

It occurred to us that the relative inagglutinability of the blood-cultures might be due to the presence of some protective substance produced in the growth of these organisms. An attempt to prove this hypothesis by adding the supernatant extract of ground blood bacilli to agar-cultures resulted negatively: the agar-bacilli so treated were clumped as well as untreated bacilli by the antiagar-culture serum.

Absorption experiments have led hitherto to rather confusing results, the differences depending in the main on employment of more or less agglutinable strains of the bacillus. We prefer to reserve expression of opinion on this point to a later communication.

The practical interest of the relative inagglutinability of blood-strains of *B. typhosus* lies in its applicability of recently isolated strains of the micro-organism. In our preliminary communication,<sup>7</sup> we expressed the belief that freshly isolated typhoid bacilli which failed to respond to the characteristic serum test might respond more readily to an immune serum "obtained by immunizing animals with cultures grown on rabbit

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5. Carpano: Ueber die Kapsehulle einiger Bakterien. *Centralbl. f. Bakteriol.* 1913. lxx, 42.

6. We are indebted to Miss Agnes Scholl for certain of these observations.

7. Gay and Claypole: Induced Variations in the Agglutinability of *Bacillus Typhosus*. *Jour. Am. Med. Assn.* 1913. lx, 1141.

(or possibly only on human) blood-agar." Such anticipation we have since been able to verify in practice.

It is a well-known fact among workers in diagnostic laboratories that cultures isolated from suspected cases of typhoid fever or from the stools of typhoid-carriers not infrequently fail at first of identification by their non-agglutinability with an antityphoid serum. Experience seems to vary as to the frequency with which such inagglutinable organisms are met. Thus Ledingham<sup>8</sup> says he has had little trouble in agglutinating fresh cultures from "carriers." Sawyer,<sup>9</sup> on the other hand, found that his eventually successful culture from a typhoid carrier on shipboard consisted in numerous colonies of organisms that biologically agreed with the characteristics of *B. typhosus*, but could not be finally identified by an agglutination test until they had been subcultured for ten to twelve generations on agar. Courmont and Rochaix<sup>10</sup> have studied hundreds of typhoid strains isolated from blood and stools of typhoid patients and also from dogs that had been given the dejecta of such patients. They state that many of these organisms are at first little agglutinable and do not become so until they have been subcultured in bouillon for ten to twelve generations.

Schiller<sup>11</sup> has found great variation in the agglutinability of suspected typhoid bacilli isolated from stools, some organisms from a given stool agglutinating readily with a strong antityphoid serum (titer 1 to 8,000), while others from the same stool failed to be clumped in a dilution of 1 to 100.

Kolle and Hetsch<sup>12</sup> state that very few real typhoid bacilli fail to agglutinate entirely, but that many agglutinate only feebly. Paltauf<sup>13</sup> states that the well-known failure of recently isolated typhoid cultures to agglutinate at best delays diagnosis and frequently leads to error.

Raubitschek and Natonek,<sup>14</sup> in a recent comparative study of strains of typhoid bacilli, express doubt of the absolute identity of some of their strains in view of the fact that some of them fail to agglutinate with a

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8. Ledingham: The Carrier Problem in Infectious Diseases. Longmans, Green & Co., London, 1912.

9. Sawyer: A Typhoid Carrier on Shipboard, Jour. Am. Med. Assn., 1912, lviii, 1336, and personal communication.

10. Courmont and Rochaix: Technique de la détermination du bacille d'Eberth par la recherche de l'agglutination. Compt. rend. Soc. de biol., 1910, lxi, 134.

11. Schiller: Beiträge zur Typhus-Epidemiologie. Centralbl. f. Bakteriologie, 1908, xvi, 385.

12. Kolle and Hetsch: Die experimentelle Bakteriologie, etc., Urban and Schwarzenberg, Berlin, edit. 3, 1911, p. 261.

13. Paltauf: Die Agglutination. Handbuch der pathogenen Micro-organismen, Kolle and Wassermann, Ed. 2, 1912, ii, 505.

14. Raubitschek and Natonek: Ueber Unterschiede in den biologischen Eigenschaften der Typhusbacillen. Centralbl. f. Bakteriologie, 1913, I Abt., orig., lxi, 241.

strong antityphoid serum. It is interesting to note that their cultures have all been recently isolated from various organs of cadavers.

We have of late had the opportunity of examining three recently isolated typhoid cultures, one of which (D) was reported as failing to agglutinate with an antityphoid serum.

TABLE 2.—AGGLUTINATION OF FRESHLY ISOLATED STRAINS OF *B. TYPHOSUS* IN VARIOUS GENERATIONS WITH ANTIAGAR AND ANTIBLOOD IMMUNE SERUM FROM THE RABBIT\*

<i>B. Typhosus</i> Culture Generations on Agar	Antiagar Agglutinating Serum 5†	Antiblood Agglutinating Serum 42
Stock 5 .....	1 : 16,000 +	1 : 2,000
A 1 .....	1 : 1,000	1 : 500
B 2 .....	1 : 100	1 : 2,000
C 2 .....	1 : 10,000 +	1 : 2,000
D 2 .....	1 : 100	1 : 500
D 5 .....	1 : 100	1 : 2,000
D 8 .....	1 : 1,000	1 : 2,000
D 9 .....	1 : 1,000	1 : 2,000
D 12 .....	1 : 2,000	1 : 2,000

\* Cultures tested: (A) From Dr. J. V. Cooke, University of California Hospital. Original culture from spleen (necropsy 1337, May 24, 1913). Kept on ice without subculture from May 27 to June 12; (B) From Dr. J. V. Cooke. Cultures from blood of patient furnishing culture A taken three days before death. One transplantation. Kept on ice from May 23 to June 12; (C) From Dr. J. V. Cooke. Blood-culture from Mrs. N. taken May 13, 1913. Second transplant. Kept on ice from May 15 until test June 12; (D) From Dr. E. Foster. These cultures were tested with both an antiagar culture typhoid serum from rabbit (5) and an antiblood culture typhoid serum from rabbit (42); the least agglutinable culture was repeatedly sub-cultured at twenty-four-hour intervals on agar until it became readily agglutinable by the antiagar as well as the antiblood serum.

† The plus sign indicates that the limit of agglutination was not reached in the given experiment.

Table 2 shows that the antiagar-culture serum 5 is the more potent, agglutinating the Stock Culture 5 in a dilution of 1 to 20,000 (Table 1). The antiblood-serum agglutinates the stock organism in a dilution of 1 to 2,000 only. Of the freshly isolated cultures, one only, C in the second transplantation, reacts like a stock culture of *B. typhosus* to an antiagar or the usual diagnostic serum. Culture A reacts relatively feebly to the antiagar serum so that diagnosis might be questioned. Cultures B and D react only in a dilution of 1 to 100, and it must be remembered that they were used first when in the second generation on agar. Even in this generation their diagnostic identification as *B. typhosus* could not be accepted. But the point of most interest is that all the organisms react strongly with the absolutely weaker antiblood-culture serum. The reaction of Culture B2 is particularly striking—diagnosis negative or at best doubtful with the antiagar-serum, but strikingly positive with antiblood-

serum, 1 to 2,000. The subsequent generations of D show how the growth on agar renders the inagglutinable organism susceptible to an ordinary rabbit immune serum obtained with agar-cultures.

The conclusion seems justified that the diagnosis of suspected typhoid bacilli can be rendered immediately certain even on first isolation by the use of an immune serum obtained by treating animals with cultures of the organism grown on blood. The application of this diagnostic procedure to other organisms such as *B. dysenteriae* (particularly of the Shiga type) would be interesting.



## THE EXAMINATION FOR DIAGNOSTIC PURPOSES OF THE ENZYME ACTIVITY OF DUODENAL CONTENTS \*

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Since the introduction of the duodenal tube by Einhorn<sup>1</sup> and by Gross,<sup>2</sup> this method of reaching the duodenum has been employed for a variety of purposes. It has been used to maintain nutrition in diseases of the stomach and other conditions,<sup>3</sup> to treat a variety of constitutional diseases by irrigation<sup>4</sup> and to secure fluid for a study of the bacterial flora of the upper intestine, and likewise the activity of the pancreatic enzymes and the detection of bile. Despite the many possibilities which have been opened by this method of entrance into the intestines, it would appear to have received attention only in a few circles. As a method of ascertaining the activity of the pancreas for diagnostic purposes, it is obviously more logical than the various indirect methods entailing an examination of the feces or urine.

The results which have thus far been obtained in the examination of duodenal contents deserve brief review. It has been shown by Hess<sup>5</sup> in infants, and more recently by MacNeal and Chace<sup>6</sup> in adults, that this method may be employed to excellent advantage in the study of the bacterial flora of this part of the intestines. MacNeal and Chace observed that, normally, when free hydrochloric acid was present in the stomach, the contents of the duodenum were comparatively sterile, but when free acid was absent, numerous organisms were present. In a case of typhoid which they had the opportunity to examine, typhoid bacilli were present in large numbers, while in an infant, aged 22 months, Hess made a similar observation.

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\* Submitted for publication Sept. 20, 1913.

\* From the Medical Service and the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital.

1. Einhorn: *Med. Rec.*, New York, 1910, lxxvii, 98.

2. Gross: *New York Med. Jour.*, 1910, xci, 77.

3. Einhorn and Rosenbloom: *Am. Jour. Med. Sc.*, 1911, cxlii, 7; Einhorn: *Post-Graduate*, 1913, xxviii, 550; Hess, A. F.: *The Use of a Simple Duodenal Catheter in the Diagnosis and Treatment of Certain Cases of Vomiting in Infants*, *Am. Jour. Dis. Child.*, 1912, iii, 133; Morgan: *Am. Jour. Med. Sc.*, 1912, cxliii, 670.

4. Gross: *New York Med. Jour.*, 1911, xciii, 171; *Med. Rec.*, 1912, lxxxix, 706; Jutte: *New York Med. Jour.*, 1912, xcv, 543.

5. Hess, A. F.: *Jour. Infect. Dis.*, 1912, xi, 71.

6. MacNeal, Ward J., and Chace, A. F.: *A Contribution to the Bacteriology of the Duodenum*, *THE ARCHIVES INT. MED.*, 1913, xii, 178.

The first paper of importance on the enzyme activity of the duodenal contents was that of Einhorn and Rosenbloom,<sup>7</sup> who studied the influence of various agents on the secretion and activity of the juice in a series of persons. In general, they were able to confirm the physiologic observations on animals. They noted that the secretion was active during fasting, while the administration of secretin, hydrochloric acid, pilocarpin, etc., caused an increased secretion of fluid, and generally a more active one. The activity of the juice in diseases possibly involving the pancreas was not considered.

Several very instructive papers on the examination of the juice in infants have appeared by Hess,<sup>8</sup> who conducted much of his work in the baby wards of this hospital. He observed that bile was rarely excreted during the first twelve hours of life, while in the subsequent twenty-four hours it was variable. In cases of marked jaundice it was observed to be profuse, the jaundice preceding the excretion of bile into the duodenum. The three pancreatic ferments were found in the infants before they had been put to the breast, and thus without the stimulus of food to incite the secretion. In older infants, a month or more of age, with the increase in the secretion of the juice, a decided increase in the amylolytic power was observed. The development of this starch-splitting enzyme so early in life is particularly interesting. In certain atrophic infants it was noted that, although they secreted little gastric juice to act as a stimulus, a very large amount of thin, watery juice containing all the pancreatic ferments, though weak in lipase, could be aspirated from the duodenum.

In a series of cases of adults without pancreatic disease, Frank<sup>9</sup> found active trypsin in all cases.

While the present work was in progress an interesting paper appeared by Crohn<sup>10</sup> on the diagnosis of the functional activity of the pancreatic gland by means of ferment analyses of the duodenal contents. His series included a number of cases of interest, namely one case of acute pancreatitis, three cases of obstructive jaundice, five cases of cholelithiasis and six of diabetes. In the case of pancreatitis, amylase and trypsin were found absent from the duodenal juice and likewise from the feces. The cases of obstructive jaundice showed an absence of bile, but a normal enzyme activity, except in one case, in which the amylase and trypsin

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7. Einhorn, Max, and Rosenbloom, Jacob: A Study of the Duodenal Contents in Man, *THE ARCHIVES INT. MED.*, 1910, vi, 666.

8. Hess, A. F.: A Study of Icterus Neonatorum by Means of the Duodenal Catheter, *Am. Jour. Dis. Child.*, 1912, iii, 304; The Pancreatic Ferments in Infants, *Am. Jour. Dis. Child.*, Oct., 1912, iv, 205. A Consideration of the Pancreas and Its Ducts in Congenital Obliteration of the Bile-Ducts, *THE ARCHIVES INT. MED.*, 1912, x, 37.

9. Frank: *Arch. Verdauungskr.*, 1912, xviii, 387.

10. Crohn: *Am. Jour. Med. Sc.*, 1913, cxlv, 393.

were absent on the first examination. The duodenal juice in the cases of cholelithiasis and of diabetes was found normal. This observation in diabetes would appear to emphasize the point, sometimes misunderstood, that there is no disturbance of the external secretion of the pancreas in this condition.

A study of the enzyme activity of duodenal juice was begun more than a year ago. The objects of the investigation were (1) to establish the normal limits of variation in the activity of the pancreatic enzymes, (2) to ascertain the chemical composition and enzyme activity of the duodenal juice in a variety of pathologic conditions, (3) to determine what influence the gastric acidity might exert on the composition of the duodenal juice, and (4) to perfect methods of determining the admixture of bile by the estimation of the bile pigments and cholesterol. In view of the scant attention which the problem has attracted, we have thought it desirable to report the results which have been obtained in the examination of duodenal juice in thirty cases. It is planned to continue the study on interesting cases as they may arise.

#### METHOD OF OBTAINING DUODENAL JUICE

The technic employed in obtaining the duodenal juice has already been described by MacNeal and Chace.<sup>11</sup> A small soft rubber tube (external diameter of 3.5 mm.) capped with a perforated gold tip, is placed in the patient's mouth with instructions to swallow. With the patient lying on his right side, the tube is usually carried by the peristaltic waves of the stomach into the duodenum in about twenty minutes. Its position can be demonstrated either fluoroscopically or by examination of the aspirated contents. The tube is usually allowed to pass to about 80 cm. It was generally given at 10:30 p. m. and the duodenal contents aspirated with a glass syringe at about 9:00 a. m.; then with the tube still in place an Ewald test-breakfast was given and the duodenal contents aspirated one hour later. Occasionally the tube was passed at 7 a. m. and the duodenal contents aspirated at 9 a. m. This method is generally as accurate, and is more acceptable to the patient.

*Duodenal Juice.*—The duodenal juice obtained in this way is normally a clear or nearly clear, golden yellow, slightly viscid fluid. It is faintly acid, neutral or faintly alkaline in reaction to litmus and has a specific gravity of about 1.005. The juice contains bile as its color would indicate, and active amylolytic, lipolytic and proteolytic enzymes.

*Methods of Examination.*—The methods which have been employed in the examination of the duodenal juice are briefly described below. They are slight modifications of well-known methods which have been simplified in so far as possible for use in this connection. It was apparent in this work that the determinations of the enzyme activities were only relatively quantitative. On this account, and because of the small amounts of fluid often obtainable, a relatively simple technic has been employed.<sup>12</sup> With the technic described, complete data can be secured with 6 to 7 c.c. of juice.

11. MacNeal, Ward J., and Chace, A. F.: A Contribution to the Bacteriology of the Duodenum, *THE ARCH. INT. MED.*, 1913, xii, 178.

12. These methods have been described by Myers and Fine: *Essentials of Pathological Chemistry*, New York, 1913, p. 25.

After securing the juice, it was taken to the laboratory without delay and placed in a refrigerator at 0 C., unless the examination was to be made at once. In the latter half of the present series this was the case. It is not believed, however, that refrigeration, at least for a short period, produces any appreciable change in the enzyme activity of the juice. It was very soon observed that little or no amylolytic activity could be demonstrated in a juice which was slightly acid to litmus, even though the juice had been at once neutralized when received. The proteolytic activity was affected in the same way, though to a much less extent. In the case of the lipase it was observed that the juice, which was faintly alkaline to litmus, always showed a weak lipolytic activity. It should be noted here, however, that the juice was not previously acidified. The initial reaction of the juice is obviously an important factor in the observed enzyme activity.

(a) *Reaction and Total Acidity*.—The reaction was ascertained with strips of red and blue litmus, while the total acidity was titrated with N/20 NaOH, using phenolphthalein as indicator, and employing as large amounts of material as might be available.

(b) *Amylase (Amylopsin)*.—The Wohlgemuth method is simple and fairly satisfactory. Into each of six small test-tubes are introduced 5 c.c. of 1 per cent. soluble starch solution. Tube one serves as a control and to the remaining five tubes are added 0.05, 0.1, 0.25, 0.5, and 1.0 c.c. of the juice diluted one-half with distilled water. The tubes are then incubated at 38 C. for thirty minutes, immediately nearly filled with cold water, two drops of N/10 iodine added, and the tubes shaken. The tube is selected as positive which shows an entire disappearance of all blue color. The enzyme activity is expressed in the number of c.c. of starch solution 1 c.c. of undiluted juice is capable of digesting. If it takes 1 c.c. of juice to digest 5 c.c. of starch, the activity is five; if digestion is accomplished by 0.25 c.c., it is twenty, etc. For the five tubes as diluted above, the activity figures are 200, 100, 40, 20 and 10.

(c) *Lipase (Steapsin)*.—Into each of two test-tubes are introduced 1 c.c. portions of the juice, one of which is boiled to serve as control. To each of these tubes are added 1 c.c. of neutral ethylbutyrate, 10 c.c. of distilled water and 1 c.c. of toluene. The tubes are shaken and placed in the incubator at 38 C. for twenty-four hours, shaking several times during the interval. At the end of this time they are removed to porcelain dishes and titrated with N/20 NaOH, using phenolphthalein as indicator. The titration result of the boiled tube is subtracted from the unboiled to obtain the figure for the lipolytic action.

(d) *Protease (Trypsin)*.—Two methods have been employed, the O. Gross casein method, and a modification of the Fermi gelatin method. Casein has the disadvantage that it is also attacked by erepsin, though this is probably a negligible factor here, while the gelatin digestions must be carried on at room temperature.

*Casein Method*.—Into each of six small test-tubes, as in the amylase method, are introduced 5 c.c. of 0.1 per cent. pure casein in 0.1 per cent. sodium carbonate,<sup>13</sup> and the same amounts of duodenal juices added as in the case of the amylase. The tubes are incubated for fifteen minutes at 38 C. and then acidified with two drops of dilute acetic acid. The tube which remains perfectly clear, that is, in which digestion has been complete, is recorded. The tryptic activity is calculated in the same way as the amylolytic activity, except that the tryptic activity, according to the Gross formula, is an expression of the power of 1 c.c. of the juice on 10 c.c. of the casein solution, instead of 1 c.c., as in the Wohlgemuth method. The five tubes, according to the Gross scheme, represent activities of 20, 10, 4, 2 and 1, that is, one-tenth of the values obtained in the case of the amylase.

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13. The soluble starch and casein solution were made up a liter at a time, a little chloroform and toluene added, and kept in a refrigerator at 0 C.



ENZYME ACTIVITY OF DUODENAL CONTENTS TABULATED ACCORDING TO GASTRIC ACIDITY  
GROUP I. CASES SHOWING AN ABSENCE OF FREE HYDROCHLORIC ACID

Case	Diagnosis	Gastric Acidity		Total Duodenal Acidity	Volume of Fluid c.c.	Con- dition §	Reaction to Litmus	Amy- lase	Trypsin	
		Free HCl	Total Acidity						Casein	Gelatin em.
1—B. A. ....	Myxedema .....	0	6	5	14	F	Neutral .....	5	2	3.9
2—M. McG. ...	Chr. gastric ulcer.	0	8	8	19	F	Neutral .....	200	4	3.7
3—J. K. ....	Asthenic gastritis.	0	10	10	5	F	Neutral .....	20	4	3.8
4—J. L. ....	Pernicious anemia	0	20	9	20	F	Neutral .....	10	2	5.6
	..	..	..	..	15	F	.....	100	4	4.2

GROUP II. CASES SHOWING AN HYPERACIDITY, THE FREE HCl BEING ABOVE 25

5—I. S. ....	Gastric ulcer .....	50	77	12	20	F	Neutral .....	100	4	3.2
	..	..	..	10	6	E	Faintly alk...	100	10	2.1
	..	..	..	10	7	F	Faintly alk...	10	10	4.2
6—W. L. ....	Gastric ulcer ....	50	70	5	6	E	Neutral .....	100	4	4.3
7—C. L. ....	Gastric ulcer .....	50	75	..	5	F	Faintly alk...	100	4	3.1
	..	..	..	23	15	E	Faintly acid..	5	0	3.2
	..	..	..	25	6	E	Faintly acid..	0	0	5.3
8—M. P. ...	Hyperchlorhydria	30	70	10	10	F	Neutral .....	200	4	3.9
	..	..	..	..	2	E	Neutral .....	...	...	3.9
9—R. L. ....	Alcoholic gastritis..	28	58	55	15	F	Acid .....	0	0	0.4
	..	..	..	75	13	E	Acid .....	0	2.3	...
	..	..	..	85	10	F	Acid .....	0	0	...
	..	..	..	65	27	E	Acid .....	..	2.7	...
	..	..	..	35	10	F	Acid .....	...	1.5	4.2
	..	..	..	80	24	E	Acid .....	...	1.3	1.0
	..	..	..	80	25	E	Acid .....	...	2.8	0.7
10—S. W. ...	Neurosis .....	30	60	15	9	F	Faintly acid..	10	4	4.9
	..	..	..	10	27	F	Neutral .....	20	10	5.2
	..	..	..	..	4	E	Neutral .....	10	4	4.2
	..	..	..	..	23	F	Faintly acid..	0	2	3.5
11—T. B. ....	Duodenal ulcer ...	73	89	5	5	F	Faintly alk...	10	2	2.6
	..	..	..	15	15	E	Neutral .....	5	...	3.4
12—A. B. ....	Gastric ulcer .....	90	105	0	40	F	Alkaline .....	2	10	4.8
	Gastro-enterostomy	..	..	0	20	E	Alkaline .....	40	10	4.5

13—B. B. ....	10	35	10	17	F	Neutral	40	0.3	10	2.8
	..	..	..	6	E	Acid	0	..	2	0.6
14—A. P. ....	21	60	35	15	F	Faintly acid..	0	1.7	0.5	1.7
15—G. H. ....	10	37	8	13	F	Faintly alk...	40	2.1	10	5.8
	..	..	13	10	E	Faintly alk...	40	2.3	10	4.7
16—B. D. ....	15	50	15	6	F	Faintly alk...	100	1.9	4	3.3
17—F. S. ....	20	35	13	15	F	Faintly acid..	5	3.1	4	3.7
	..	..	25	12	E	Faintly acid..	2	3.8	2	3.0
18—C. F. ....	30	54	10	10	F	Neutral	10	1.7	4	3.4
19—E. H. ....	24	56	5	10	F	Neutral	5	2.0	2	3.7
20—R. E. ....	13	27	10	8	F	Neutral	..	0.9	..	3.6
	..	..	5	8	E	Neutral	..	0.9	..	3.1
21—A. O'C....	8	16	..	3	F	Faintly alk...	40	..	4	3.6
	..	..	..	2	F	Faintly alk...	100	..	2	..
	..	..	5	19	F	Faintly alk...	100	0.6	4	4.4
	..	..	8	7	F	Faintly alk...	200	0.4	4	3.6
	..	..	8	9	F	Faintly alk...	40	2.2	4	4.5
	..	..	45	8	*	Neutral	100	2.0	4	5.4
22—S. Z.†....	17	53	..	5	F	Faintly acid..	0	..	4	3.1
	..	..	20	11	F	Faintly acid..	0	1.8	0.5	2.6
	..	..	15	9	F	Faintly acid..	0	1.6	2	3.2
	..	..	..	5	F	Faintly acid..	0	1.3	0.5	1.4
23—S. S. ....	26	46	..	1	F	Faintly alk...	0	..	0	..
	..	..	4	26	F	Faintly alk...	0	0.6	0	0.0

GROUP IV. MISCELLANEOUS CASES

24—J. F. ....	..	..	20	12	F	Faintly alk...	40	2.9	10	3.0
	..	..	15	15	E	Faintly alk...	100	1.2	10	3.4
25—W. D. ...	..	..	3	10	F	Neutral	40	0.3	3	3.0
	..	..	3	3	E	Neutral	100	..	2	3.5
26—M. G. ...	12	30	5	10	F	Neutral	10	1.9	10	5.3
	0	12	5	8	E	Neutral	20	1.8	10	4.5
27—L. L. ....	..	..	10	20	F	Neutral	40	0.8	20	5.4
	..	..	10	10	E	Neutral	2	1.3	4	4.3
28—A. N. ...	14	14	5	10	F	Neutral	40	0.4	4	2.8
	..	..	..	2	E	.....	..	..	..	2.6
29—S. G.†....	..	..	10	10	F	Neutral	100	0.1	1	4.3
30—J. O. ....	7	32	7	16	F	Faintly alk...	100	1.8	10	4.5

\* Test meal of milk, cream and boiled starch. † Bile absent from duodenal juice on each examination. ‡ Bile present in duodenal

*Gelatin Method.*—Into a small test-tube are pipetted 1 c.c. of undiluted duodenal juice, 3 c.c. of water, 1 c.c. of 0.5 per cent. sodium carbonate and 1 c.c. of toluene. Into the test-tube is then inserted two 3 cm. gelatin tubes,<sup>14</sup> and the test-tube allowed to incubate for forty-eight hours at room temperature with occasional gentle mixing. At the end of this time the amount of digestion is measured with a millimeter scale and the measurements added together.

(c) *Bile.*—The test employed for bile has been the simple Gmelin nitric acid test, though ordinarily sufficient bile is present to make a qualitative test unnecessary. In future analyses we plan to employ modifications of the Huppert test for bile pigments, and the Liebermann-Burchard test for cholesterol as the basis of colorimetric methods for estimating these substances.

The subjects of the present study were adults of an average age of about 35 years, sixteen being males and thirteen females.

#### DISCUSSION OF RESULTS

As may be observed from the tabulated data, the activities of the pancreatic enzymes show some little variation from day to day under normal conditions. Similar observations were made by Einhorn and Rosenbloom and by Crohn. The greatest variation was noted in the amylase and lipase, and was apparently dependent in part on the initial reaction of the juice. The favorable reaction for these two enzymes is quite opposite, neutral or faintly alkaline for amylase and faintly acid for lipase. The activity of the trypsin was more constant. In Patient 21 — A. O'C. — the tryptic activity was quite uniformly 4 over a period of several days, whereas the activity of the amylase varied from 40 to 200, and that of the lipase from 0.6 to 2.2. In the entire series, the amylolytic activity ranged from 5 to 200, the lipolytic activity from 0.3 to 3.8 and the proteolytic from 0.5 to 10 (casein method) and 1.4 to 5.6 (gelatin method).

It may be noted in Case 4 — J. L. — with pernicious anemia, that though there was an almost complete absence of gastric juice, the duodenal juice was very active, especially the trypsin (gelatin test). In Case 29 — S. G. — with infective jaundice, the lipolytic activity was very low, 0.1. The activity of the pancreatic enzymes was normal in the case of hypertrophic cirrhosis of the liver, 25 — W. D. In Group II, Case 9 — R. L. — with alcoholic gastritis, the juice was found to be strongly acid. This was believed at first to have been due to an error in technic, but the active lipase and the good digestion of gelatin on the one occasion when the acidity was comparatively low, would indicate otherwise. The results suggest an inability on the part of the pancreatic juice to neutralize the acid of the gastric juice. The results in Case 12 — A. B. — with gastric ulcer (gastro-enterostomy), are in striking contrast. Here the free gastric

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14. The gelatin tubes were prepared by dissolving 10 gm. of gelatin and 1 gm. of sodium fluorid in boiling distilled water, deeply coloring with a clear solution of cochineal, making up to 100 c.c. and then filling glass tubing of 2 mm. inside diameter. The tubes 3 cm. in length are cut just previous to use.

acidity was 90 and the total acidity 105. The duodenal juice in this case was decidedly alkaline to phenolphthalein. The lipolytic activity was weak, but the proteolytic activity very strong. When this is considered, together with the large volume of juice obtained, it would appear to indicate that the pancreas had responded to the stimulus of the high gastric acidity. From the tabulated data it seems evident, however, that the acidity or lack of acidity of the gastric juice is without definite influence on the enzyme activity of the pancreatic juice, although a strongly acid gastric juice is apparently able in most cases to stimulate the production of a sufficiently alkaline duodenal secretion to neutralize it.

The normal variation in the activity of the pancreatic enzymes is so great, as evidenced by the data tabulated above, that only an absence of the enzymes would appear to render the juice of diagnostic value. The absence of pancreatic enzymes in the duodenal juice, would, however, be positive evidence of either pancreatitis or of non-potency of the pancreatic ducts, while the lack of bile in the juice would be pathognomonic of the occlusion of the common bile duct. By means of an analysis of the duodenal juice, the surgeon should in certain cases be able to determine the involvement or non-involvement of the pancreas prior to operating on the gall-bladder or gall-ducts.

In the case of chronic pancreatitis, 23 — S. S. — the enzyme activity of the duodenal juice was negative, except for 0.6 of lipase. The reaction of the juice was favorable for the detection of amylase and trypsin — faintly alkaline — and sufficient material, 26 c.c., was available for very careful examination. The stools were typical of pancreatitis. This coincides with the results obtained by Crohn in his case of pancreatitis, though it should perhaps be noted that there the fluid was acid.

In the case of carcinoma of the gall-ducts and the pylorus, 2? — S. Z. (diagnosis confirmed by operation) — the pancreatic ducts were not involved as shown by the active enzymes. (The absence of amylase was probably due to the reaction of the juice, faintly acid.) Bile could not be detected in any of the four specimens obtained.

#### CONCLUSIONS

Active amylolytic, lipolytic and proteolytic enzymes are present in duodenal juice, though the activity of these enzymes is apparently subject to considerable variation under normal conditions.

The acidity of the gastric juice appears to be without influence on the activity of the enzymes present in the duodenal juice.

In a case of carcinoma of the gall-ducts and pylorus with biliary obstruction, there was an entire absence of bile from the duodenal juice. In a case of chronic pancreatitis, the amylolytic and proteolytic activity was entirely negative, while the lipolytic activity was comparatively weak.



The absence of pancreatic enzymes from the duodenal juice would appear to be positive evidence of either pancreatitis or non-potency of the pancreatic ducts, while the lack of bile would appear to afford similar evidence of the occlusion of the common bile duct. Further observations on these conditions are desirable.

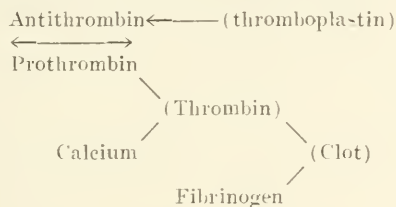
We desire to express our obligation and thanks to Dr. Edward Quintard, Director of the Department of Medicine, for permission to use the clinical material, and to Drs. B. Lattin and W. G. Lough for personal attention to details in collecting the specimens from the patients.

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## II. HEMORRHAGIC DISEASE. ANTITHROMBIN AND PROTHROMBIN FACTORS \*

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In a previous communication dealing with various types of hemorrhagic disease (Whipple<sup>1</sup>), we suggested that one factor, namely, antithrombin, might be of importance in various types of bleeding. The other elements of blood coagulation were taken up and some of the work on normal blood coagulation was reviewed. Because it is of great importance to have clearly in mind the mechanism of normal blood coagulation, it may be well to detail briefly Howell's<sup>2</sup> theory, as it seems to meet all the known requirements in the most satisfactory manner.



This shows in a schematic way the manner of blood coagulation. Howell believes that a small amount of antithrombin is present in the normal plasma and is sufficient to bind the prothrombin and render it inactive. Thromboplastin is set free by cell injury (blood-cells, platelets, tissues, cells, etc.), and neutralizes the antithrombin. This frees the prothrombin, which at once combines with calcium to form thrombin. The free thrombin coagulates the fibrinogen, giving the normal clot.

It is at once evident that this balance of antithrombin and prothrombin must be extremely delicate and capable of rapid adjustment. When the antithrombin accumulates in excess, the appearance of purpura and bleeding is to be expected, but if the antithrombin drops to a low level, spontaneous thrombosis might occur.

It is not surprising then that this very point seems to be the one about which most of the interesting cases of hemorrhagic disease may be grouped.

\* From the Hunterian Laboratory of Experimental Pathology, Johns Hopkins Medical School.

\* Submitted for publication Oct. 17, 1913.

1. Whipple, G. H.: *THE ARCHIVES INT. MED.*, 1912, ix, 365.

2. Howell: *Am. Jour. Physiol.*, 1910, xxvi, 453.

## METHOD

Blood was obtained in all cases before death by means of a needle in a vein. The blood was aspirated into a solution of sodium oxalate (1 per cent.), the dilution being nine parts blood to one of oxalate. The blood from autopsy cases was usually obtained directly from the heart by means of a trocar and drawn into a bottle containing oxalate. Clear plasma was obtained by centrifugalization. In all experiments the calcium was supplied in the form of 1 per cent. calcium chlorid solution. The coagulation experiments were always done with the fresh blood plasma unless otherwise stated and the observation usually completed within two to six hours after the blood had been obtained. Control plasma from normal men, cats or dogs was always used fresh. Spleen extract was used only when fresh and made up by grinding the spleen with sand to a thin paste, adding sufficient salt solution and filtering through paper.

## ICTERUS, LIVER DISEASE, BLEEDING

CASE 1.—Carroll. White male, aged 75. Admission July 11, 1912. Death July 20, 1912. Complaint, jaundice.

Family history negative. Past history negative, except strong alcoholic history up to 17 years ago.

P. I. Began April 19, 1912, with sudden appearance of jaundice which has been continuous ever since. On admission, skin was of a deep bronze color. The liver was definitely enlarged. There were marked hemorrhoids. Urine rich in bile. The general clinical picture suggested the diagnosis of cancer of the head of the pancreas.

July 12. White blood cells 12,100. Hb. 82 per cent. Stools contained no blood and were of acholic type. No evidence of bleeding.

July 13. No evidence of bleeding. Intense icterus. Blood drawn directly into oxalate from arm vein. (Table 1.)

TABLE 1.—CASE 1. COAGULATION TIME OF BLOOD IN JAUNDICE

Plasma C c.c	Plasma H c.c	Calcium Solution Drops	Coagulation Time Minutes
1	..	3	8
1	..	4	8
1	..	2	No clot
..	1	3	4
..	1	5	5

Plasma H is from a normal man. The slight delay of coagulation (Carroll) comes nearly within normal limits but may in view of later developments indicate a slight excess of antithrombin.

July 17. Red blood-cells 3,500,000. Hb. 70 per cent. W. B. C. 12,400. *Bleeding* was first noticed. There was oozing from the mouth, and blood-stained feces. Patient was in a low delirium.

July 18. Patient bleeding from mouth and there is some blood-stained vomitus. Blood is present in the stools and patient appears sick and is losing ground rapidly.

2:30 p. m. Blood drawn directly from vein into oxalate. This plasma showed an excess of antithrombin. (Table 2.)

TABLE 2.—CASE 1. SECOND EXPERIMENT

Plasma C c.c.	Plasma D c.c.	Calcium Solution Drops	Coagulation Time Minutes	
			Start	End
1	..	3	14	40*
1	..	5	17	60*
..	1	4	3	3
2	1	15	3½	8
..	1	4	3	4
3	1	13	4½	11
1	..	2	26	..
1	..	1	26	..
1	1	7	5½	8-11*

\* Clots wiped out often and subsequent clots formed slowly up to the final time. Plasma D obtained from a normal dog.

July 19, 9 a. m. The plasmas used on previous day were kept on ice. Fresh spleen extract made as usual. (Table 3.)

TABLE 3.—CASE 1. THIRD EXPERIMENT

Plasma C, c.c.	Plasma D, c.c.	Spleen Extract Drops	Calcium Drops	Coagulation Time, Minutes	
				Start	End
1	..	..	3	35	..
1	..	4	3	3½	8-12
1	..	5	3	3½	6
..	1	6	5	1	..

Fresh spleen extract is rich in thromboplastin which can neutralize antithrombin with rapidity. It overcomes the slight amount of antithrombin in normal plasma and clots it in one minute, while the coagulation time of the antithrombin plasma is lowered to one-tenth its control. (Table 3, Case 1.)

July 20, 1 a. m. Death took place. Patient had been in a semistupor for the previous twelve hours. Temperature had been constantly subnormal.

1:20 a. m. Blood obtained directly from auricle and drawn into oxalate. The tests given below were performed immediately. Analysis shows the excess of antithrombin to have disappeared in great part, while the prothrombin has greatly diminished in amount. (Table 4.)

1. Blood drawn into clean bottle (no oxalate) began to clot slightly at edges after one and one-half hours; after twelve hours a flabby clot had formed all through the blood. After thirty-six hours in the ice box a tough clot had formed with retraction and escape of serum. There was no evidence of clot autolysis after three days.

2. Plasma C,<sup>1</sup> blood drawn into a clean bottle (no oxalate) and a few minutes later centrifugalized. Plasma a dirty orange yellow, slightly opalescent and rich in bile pigments. This plasma kept in a beaker free from blood cells showed a few filmy clots in one and one-half hours and thin clots on the wall of the



vessel after twelve hours, the center being free from clots. After twenty-six hours coagulation was not complete. At the end of three days a firm clot was formed.

3. Plasma C,<sup>2</sup> blood (Carroll) drawn into oxalate, centrifuged and preserved as usual—no clots formed.

4. Plasma D, oxalate plasma from a normal dog.

5. Spleen extract from the same dog, about twelve hours postmortem.

TABLE 4.—CASE 1. FOURTH EXPERIMENT

Plasma C, <sup>1</sup> No Oxalate c.c.	Plasma C, <sup>2</sup> Oxalated, c.c.	Plasma D, Oxalated, c.c.	Spleen Extract Drops	Calcium Drops	Coagulation Time, Minutes	
					Start	End
1	.....	.....	.....	.....	30-60	
1	.....	.....	.....	2	40	
1	.....	.....	5	.....	35	50
1	.....	.....	10	.....	35	60
1	.....	*1	.....	.....	12	30+
.....	.....	1	7	5	1½	1½
1	.....	1	7	5	1½	1½
1	.....	.....	5	2	14	23
2	.....	1	.....	5	4	6
.....	1	.....	.....	5	50	.....
.....	1	*1	.....	3	6	

\* Dog serum, which was twelve hours postmortem.

From an analysis of Table 4 we may conclude that the excess of antithrombin has completely disappeared from this blood, leaving only a small amount which may be within normal limits. The prothrombin has been greatly diminished, but has fallen not quite to zero, as the blood will clot in hours or days. This blood does not inhibit normal plasma coagulation. Spleen extract causes some acceleration of coagulation as does calcium, but the great deviation from normal consists in a great drop of the prothrombin content.

The spleen from this case was tested for its thromboplastin and found to contain very little of this element. Unfortunately, spleen extracts vary greatly in their thromboplastic activity, but it is possible that this organ had been depleted of its thromboplastin, which had been used in the neutralization of the blood antithrombin.

*Necropsy No. 3754.*—Anatomical diagnosis: Carcinoma of the head of the pancreas with complete occlusion of the common bile and pancreatic ducts; extreme icterus; dilatation of bile passages and gall bladder; cirrhosis and central necrosis of liver; dilatation of pancreatic ducts with chronic pancreatitis; metastases to the nearby lymph-nodes and liver; purpura, submucous, subserous and disseminated ecchymoses; gastric and intestinal hemorrhage; pulmonary ecchymoses, hemorrhage and edema; absence of blood clots in vascular system; general arteriosclerosis; emphysema of lungs; chronic adhesive peritonitis and pleuritis; caseous bronchial lymph-nodes; hypertrophy of prostate; slight chronic nephritis.

The body is that of a large white male, 161 cm. long. Rigor mortis is present. The skin everywhere has a deep icteric hue. The pupils are equal. There are indefinite purpuric splotches about the eye, at the bend of the elbow (blood culture) on the forearm and groin and over the flanks. Liver mortis is conspicuous. On incision the subcutaneous fat is pretty well preserved and of a deep yellow color. Scattered throughout the fat, over the abdomen particularly, are little hemorrhages. The abdomen on incision contains highly colored, orange-yellow, slightly turbid fluid (500 c.c. in amount). The small intestine is moderately dilated with gas and presents a mottled purplish appearance apparently due to blood either in the mucosa or lumen. The liver appears about 5 or 6 cm. below the costal margin and is deep green in color and presents several grayish nodules apparently a new growth. The gall-bladder is huge and slightly thickened, projecting just to the edge of the liver.

Thorax: The lungs are very voluminous, cushiony and cover over the heart. There are purplish flecks and splotches in the fat over the pericardial sac, the larger ones measuring 1 cm. The pericardial cavity contains a little bile stained, bloody fluid. The cavities of the heart contain no clots, and fluid blood runs out on section. The heart weighs 380 gm. Negative except for ecchymoses.

Spleen is large and soft, weighs 200 gm., measures  $13\frac{1}{2} \times 8 \times 4$  cm. The capsule is irregular and roughened, specked with tiny ecchymoses and opaque yellow areas of thickening. There are extensive areas of thickening of the capsule near the hilum. On section the trabeculae are conspicuous. The blood-vessels stand open. The pulp scrapes off easily on the knife and is deep red, soft and velvety. The Malpighian bodies are very inconspicuous,  $\frac{1}{2}$  mm. in size.

The duodenum is intimately associated with the hard mass which appears to occupy the region of the head of the pancreas. There are extensive areas of purpura in this region. The lesser peritoneal cavity is partially obliterated by adhesions binding the firm nodular pancreas to the region of the pylorus, gastric and hepatic omentum, as well as the posterior wall of the stomach. On dissecting out the common duct it is found to be enlarged and thickened forming a tube about  $3\frac{1}{2}$  cm. in diameter, just outside of the wall of the duodenum. The enlarged gall-bladder measures  $6\frac{1}{2}$  cm. in diameter and 14 cm. in length. It is quite tense as is the common duct and distended. The cystic duct where it opens into the common duct measures about 1 cm. in diameter. The gall-bladder contains inky, black, slimy, stringy material and no calculi.

The stomach contains slimy fluid material. Its mucosa has undergone some post-mortem digestion. Ecchymoses, however, are numerous and easily made out. The orifice of the bile papilla is quite conspicuous, nodular and hard. There are two purplish projections about this papilla, one about the site of the opening of the lesser pancreatic duct. The head of the pancreas is of almost stony hardness and the growth seems to form a part of the wall which occupies the position of the bile duct. Section through the body of the pancreas shows a greatly dilated duct measuring 8 or 9 mm. in diameter. In the distal third of the organ the parenchyma is gray, translucent, very hard and firm, evidently in a condition of extreme chronic pancreatitis with atrophy. No evidence of new growth. It is embedded in a thick layer of retroperitoneal fat. About the middle part of the pancreas it seems as though some of the tissue is replaced by a new growth, the tissue being grayer and more granular than the pancreatic tissue. Section through the head of the pancreas and papilla shows a continuous growth of new tissue replacing all the normal parenchyma.

The liver weighs 2.150 gm., measures  $26 \times 21 \times 9$  cm. The surface is rather irregular, due to the presence of gray nodules, some scarcely visible, other larger ones, 2 or more cm. in diameter. The larger ones are, as a rule, associated with more or less hemorrhage. Many of them are umbilicated. They are firm to the touch. The liver tissue elsewhere has a slaty green color. On section the liver parenchyma presents a nutmeg architecture, deep green alternating with opaque gray areas, the latter being about the portal spaces. Tumor nodules of all

sizes are scattered thickly over the section. The larger ones show central degeneration and necrosis. Some of the largest show softening associated with hemorrhage and digestion of the tissue, giving rise to cavity formation in the nodule.

**Intestine:** The jejunum shows a mucosa coated with currant-red, blood-stained mucus. Ecchymoses, however, are not numerous here, and this blood in great part came from the stomach and duodenum. Low in the jejunum, however, are a few scattered ecchymoses 2 to 3 mm. in diameter in the submucous tissue. The ileum is practically free from ecchymoses except occasional ones in the submucosa. The large intestine shows a similar picture and contains a large amount of semifluid, blood-stained, fecal material. The other organs need not be described.

**Microscopical Sections.**—The lungs show hemorrhage into the alveoli with a good many polymorphonuclears, but no fibrin anywhere. In the spleen phagocytes containing old blood pigment are numerous. Marrow of femur shows little hyperplasia; phagocytes, as in the spleen, are numerous.

**Kidney:** Slight chronic nephritis. Glomeruli and tubules distended with granular, albuminous fluid. Some hemoglobin casts are found.

**Liver:** Moderate grade of portal cirrhosis with reaction of bile ducts and new formed strands of cells. The bile canaliculi are enormous and dilated with brown colloid material. The cancer nodules are associated with the portal structures. The central cells of many lobules show necrosis and there is no cellular reaction about these dead liver cells. In some areas pigmented phagocytes are crowded into the capillaries, perhaps the result of repair of preceding liver injuries.

There is abundant evidence of a progressive injury, inflammatory reaction and repair of the liver lobules. The large tumor nodules show extensive hemorrhage, but no fibrin in any section examined.

#### ICTERUS AND HEMORRHAGIC DISEASE (CASE 1)

There is much confusion about icterus and true hemorrhagic disease. The preceding case is a true example of jaundice and bleeding. It is familiar to all that a slightly delayed coagulation time is frequently associated with icterus, but such cases may be operated on with no danger and calcium will bring the coagulation time back to normal. We have suggested that this may be due to a binding of calcium by the bile pigments, a compound which is broken up with difficulty, and this renders the calcium slowly available for coagulation.

True hemorrhagic disease is rarely associated with obstructive jaundice, although the surgeons are fearful of this combination and often make a mistaken diagnosis. Calcium has no effect on this condition. For the past two years many cases of severe icterus have been examined, as a hemorrhagic tendency was suspected, but the case above is the only one of the series which showed true hemorrhagic disease.

It is of interest that we can follow the sequence of events in this case during the last week of life, while the patient developed this hemorrhagic tendency. The patient, in spite of intense icterus, of months' duration, showed no bleeding tendencies one week before death, and a blood with normal coagulation factors, except possibly a slight excess of antithrombin. Four days later true hemorrhagic symptoms appeared, which were due to an excess of antithrombin demonstrated in the blood.

The blood obtained a few minutes after death showed only a slight amount of antithrombin—perhaps a normal amount—but a great drop in prothrombin, which caused great delay in blood coagulation.

This wide fluctuation of antithrombin and prothrombin is of much interest as they may be more or less interdependent. There is some evidence that antithrombin is produced in the liver. Under certain conditions and in certain diseases (*melenia neonatorum*—see below) this element may quite disappear from the blood, but study of such cases gives no clue to the origin of prothrombin.

Here we are dealing with a disease-complex in which surely the hepatic changes are conspicuous and probably most important from the standpoint of abnormality of blood coagulation—intense and long-standing icterus with great widening of the bile canaliculi and obvious embarrassment of these liver cells; cancer metastases with rapid invasion and destruction of parenchyma; liver cirrhosis of a moderate grade with new-formed cells and abortive bile-ducts; central liver necrosis of some days duration judging from the cell-picture, yet free from the usual invasion by wandering cells. Surely this is a liver laboring under difficulties and reacting to injury in an abnormal way. There is no good reason why such a liver could not produce an excess of antithrombin. Experiments have shown that various stimulants (peptone, thrombin, etc.), which produce an excess of antithrombin in the blood, will not do so if the liver is excluded; in other words, the antithrombin excess may be produced wholly within the liver. Moreover, there is evidence from certain experiments done in this laboratory that antithrombin is being constantly formed as it is used up and is furnished in great part by the liver.

When parenchyma cells are killed in the body, we may presuppose an escape of considerable amounts of thromboplastic substance. This must be neutralized by the antithrombin or by some other agents to prevent rapid intravascular clotting. Necrosis of liver cells presumably would free an excess of thromboplastin and necessitate its neutralization. In this case we have an excess of antithrombin which vanished during the time when liver necrosis developed. May we not conclude, then, that in this instance the antithrombin excess was removed or rendered inert by the thromboplastin which escaped from the necrotic liver cells?

The terminal drop in prothrombin tempts speculation, but it may be best to refrain. At least there is a possibility that the liver is concerned in the elaboration of this substance, prothrombin, which is so elusive. The prothrombin-antithrombin balance is so delicate and its constant maintenance so important to the organism that more than one organ may be concerned in the equation, as seems to be the case with fibrinogen (Goodpasture<sup>3</sup>).

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3. Goodpasture: *Am. Jour. Physiol.*, January, 1914.



## TYPHOID (?) LIVER DISEASE (?) BLEEDING—RECOVERY

CASE 2.—Schwab. Medical No. 29,842. White male, aged 56. Admission Oct. 26, 1912. Recovery.

F. H., negative. P. H., typhoid fever at 19. Patient in bed ninety days. Various attacks of malaria for the previous fifteen years. History of moderate alcohol. Gonorrhea twenty-five years ago. Denies lues.

P. I. began October 12 with shaking chills and sweats. Since then patient has suffered from general weakness.

October 26, red blood-cells 4,448,000. White blood-cells 12,600. Hb. 75 per cent.

October 28. Widal suggestive. Blood culture negative.

October 30. Temperature has been steadily elevated between 101 and 103 F., giving a typhoid-like curve. Liver enlarged and smooth. Spleen not felt. White blood-cells 15,000. Blood smears negative. Wassermann negative.

November 2. *Bleeding* from the gums first noticed. Temperature falling gradually. Clinical diagnosis, typhoid fever.

November 3. Bleeding from gums quite marked. There are numerous subcutaneous hemorrhages. One measures 3 cm. in diameter over the deltoid. Icterus noted in the subcutaneous tissue. Red blood-cells 3,512,000. W. B. C. 10,480. Hb. 65 per cent.

November 4. Bleeding time estimated at thirty-four minutes and several hours later at eighteen minutes. Coagulation time seven minutes and forty seconds.

November 5. Stools contain blood. Bleeding from gums continuous. Jaundice quite evident. W. B. C. 9,000. Liver palpable 6 cm. below the costal margin. There are definite areas of purpura around ear pricks and abrasions in skin. Blood drawn directly into oxalate from arm vein (see Table 5). The analysis of this table shows a definite *excess of antithrombin*.

TABLE 5.—CASE 2. COAGULATION TIME IN TYPHOID FEVER

Plasma H, c.c.	Plasma D, c.c.	Serum D, c.c.	Spleen Extract Drops	Calcium, Drops	Coagulation Time, Minutes	
					Start	End
1	.....	.....	.....	5	20	*30+
1	.....	.....	.....	4	17	*30+
1	.....	.....	6	4	.....	3½
1	.....	.....	12	4	.....	2½
.....	1	.....	.....	5	.....	6
2	1	.....	.....	14	7	*20+
.....	1	.....	6	5	.....	1½
1	.....	1	.....	.....	3	.....
.....	1	1	.....	.....	3	.....

\*Clots formed slowly, wiped out and continued to reform. Plasma D, Serum D and spleen extract (weak) all fresh and obtained from a normal dog.

November 6. Purpuric spots are numerous. The gums continue to ooze blood. Widal with paratyphoid negative. Hb. 38 per cent.

November 7. Hb. 30 per cent. Bleeding stopped during the day. Blood obtained as usual tested by Dr. Howell showed normal elements of coagulation. The excess of antithrombin had disappeared.

November 12. Hb. 45 per cent. No more bleeding. Widal negative.

November 19. Hb. 48 per cent. W. B. C. 7,560. Temperature had been normal, but rose to 102.4 F. and remained elevated for ten days (relapse?). Thorax negative. Spleen palpable, but not greatly enlarged.

November 21. Blood culture and Widal negative.

November 24. Hb. 50 per cent. W. B. C. 3,400.

Dec. 22. Patient discharged in good health. Liver at this time still large and quite firm.

Clinical Diagnosis: Typhoid (?) followed by relapse. Enlarged liver.

This blood-plasma shows the characteristic slow coagulation associated with antithrombin excess. The soft clots may be removed (wiped out with a glass rod) every few minutes and other clots will reform, the process being repeated until the fibrinogen is all used up. This human plasma delayed the coagulation of normal dog plasma, in which we note again the slowly-formed clots and reformation after removal. Spleen extract which is rich in thromboplastin accelerates the coagulation to less than normal, but clots normal plasma in one and one-half minutes. It acts by neutralizing the antithrombin present. Fresh normal dog serum clots human and dog plasma alike in three minutes, indicating that this antithrombin does not retard the action of preformed thrombin on fibrinogen.

It is of interest that within a few days the hemorrhagic tendencies had vanished and blood examination revealed normal elements of coagulation. One is tempted to speculate on this transient upset of the antithrombin-prothrombin balance, and especially its *spontaneous return to normal*. As no treatment by serum, etc., was given, we are not confused by this factor, and do not hasten to credit this to the treatment. There is no reason to suppose that serum injection would be of value in such cases, as we are dealing not with a lack of any blood element, but with an excess of a normal constituent which is normally held in check by some mechanism not at present clear to us. If the antithrombin is formed by the liver and constantly used up, as some experiments indicate, it is proper to suppose that under certain conditions of liver disease or injury there may be an over-production of this substance. This case had an enlarged liver associated with an obscure infection, but unfortunately we cannot report on the anatomical changes present in the liver.

#### MILIARY TUBERCULOSIS—EPISTAXIS

CASE 3.—King. Autopsy No. 3751. Black male, aged 39. Admission June 27, 1912. Death July 12, 1912.

P. H. Patient gave history of syphilis and there was a strong alcoholic history. Patient lost weight from 149 to 100 pounds. Three months prior to admission patient bled profusely from the nose, and had such attacks with considerable frequency. Patient complained of bleeding from nose and swelling of abdomen.

P. E. Indicated probable enlargement of liver and spleen. There was no purpura. Jaundice not noted. Skin pricks bleed freely. Bleeding time seven minutes. Red blood-cells 1,900,000. W. B. C. 1,000. Hb. 26 per cent. Patient

had profuse epistaxis during the day, perhaps amounting to 200 c.c. This was checked with difficulty.

June 28. Blood aspirated from arm vein directly into oxalate. This blood plasma clotted in a normal manner in four minutes on the addition of calcium. The clot retracted to a small volume and showed no digestion after twenty-four hours. *Fibrinogen* much below normal (.083 gm. per 100 c.c.—normal 400 gm.). This suggested some liver injury.

June 30. Patient had profuse epistaxis (100 c.c.  $\pm$ ) which was checked by packing. Hb. 27 per cent. White blood-cells 1,100.

July 12. Epistaxis and definite bleeding from the gums. Urine constantly negative. Temperature showed an up and down curve from 99 to 102.5 F. Death 5:30 a. m., associated with copious hemorrhage from nasopharynx.

*Necropsy*.—No. 3751. Anatomical diagnosis: *Tabes mesenterica*; tuberculous involvement of thoracic duct; general miliary tuberculosis; acute splenic tumor; ascites.

Body is that of an emaciated colored man, 146 cm. long. The pupils are equally dilated. Rigor mortis is marked. There is edema of the ankles and feet. Abdomen is slightly distended, and half way between umbilicus and pubis there is a scar (recent paracentesis). The chest is slightly barrel-shaped. On opening the abdomen it is found to be distended with clear straw-colored fluid, but no fluid is found in the pleural or pericardial cavities. There is great enlargement of the mesenteric lymph-nodes, which vary in size from that of a marble to that of a hen's egg. They are whitish in color, firmly bound to each other and to the vertebral column. The transverse colon and one or two loops of small intestines are also tightly bound to the mass. The whole mass measures roughly 25 x 15 cm. The heart and lungs are normally placed and no adhesions of the pleura are found.

Heart negative. Lungs show congestion, edema and numerous tubercles.

Spleen weighs 620 gm. It is enormously enlarged, and retains its shape very well. The capsule is smooth, glistening and free from adhesions. It is very firm in consistence and of a dark purple color. On section the same color is observed, but it is seen to be thickly studded with great numbers of minute yellowish grains. These are small and almost invisible but are uniformly distributed throughout the whole organ. The trabeculae are not well seen, apparently being widely separated by the increase in the pulp.

Liver weighs 1,500 gm., measures 25 x 19 x 6 cm. The surface is smooth, free from adhesions, yellowish brown in color and mottled with a few dark reddish areas. On section numerous small, yellowish tubercles are found scattered throughout the whole organ. There is also a slight increase in connective tissue. The lobulation is fairly distinct. Gall-bladder normal.

*Microscopical Notes*.—Lungs filled with small areas of tuberculosis which are of the exudative type. There is little connective tissue reaction and few giant cells. Edema is well marked.

Spleen is a mass of small tubercles. The reaction is chiefly exudative, the large and small mononuclears being chiefly concerned. Necrosis and degeneration are striking. Kidney and adrenals show tubercles.

In the liver small tubercles with much necrosis are very numerous; the liver cells show much atrophy and fatty degeneration. Other organs are negative.

*Blood Examination*.—July 12, 8:30 a. m. (three hours after death) blood drawn directly from right heart into oxalate. A few filmy clots had formed, but the fibrinogen analysis showed 110 gm. per hundred c.c., which excludes coagulation of any appreciable amount. See previous analysis.

Blood drawn directly into a clean bottle with no oxalate starts to clot within thirty minutes, forming filmy masses on the side of the container. After removal of this filmy clot, secondary clots form slowly. There was some tendency towards digestion of the formed clot after twenty-four hours. Plasma D obtained from normal dog. (Table 6.)

These observations are incomplete, but indicate pretty conclusively a slight excess of antithrombin in the blood at time of death. This is one of the few cases observed by us in which there was evidence of *clot digestion* after complete coagulation. We have seen two cases in which this clot digestion or fibrinautolysis was the essential factor in the complex and probably accounted for the prolonged and fatal bleeding. More study of such cases is needed before a report can be made.

TABLE 6.—CASE 3. COAGULATION TIME OF BLOOD AFTER DEATH

Plasma H c.c	Plasma D c.c	Calcium Drops	Coagulation Time Minutes	
			Start	End
1	..	5	12	12+
1	..	5	10	12+
2	1	14	7	8
..	1	5	5	6

It is to be remembered that this case did not have outspoken symptoms of hemorrhagic disease, only periodic attacks of profuse epistaxis, and during the last day of life some oozing from the gums. The fibrinogen of the blood was quite low, and this would favor prolonged bleeding from any small wound or abrasion of the mucosa or vessels. A similar condition may be met with in liver cirrhosis with profuse gastric hemorrhage and low fibrinogen content. The blood-clots in such cases are too flabby to seal the ends of the injured vessels.

This case probably belongs in the group of antithrombin bleeders associated with a septicemia. Two weeks before death there was a normal blood coagulation, but later an excess of antithrombin appeared and was a factor in the oozing from the gums and epistaxis. The body was full of small, rapidly-growing miliary tubercles associated with much cell necrosis and tissue destruction. It may be argued that the split products from this dead protoplasm were absorbed, carried to the liver where they caused stimulation and over-production of antithrombin. The liver itself was diseased and filled with tubercles, which process of itself may have actuated this over-production.

## GENERALIZED THROMBOSIS — ANTITHROMBIN EXCESS

CASE 4.—Dean. Autopsy No. 3823. Baby born November 20 after difficult labor necessitating high forceps operation. Chloroform anesthesia thirty-five minutes. Mother recovered well. Placenta examination showed normal tissue. Weight of infant fell continuously from 4.070 to 3,130 gm. on the tenth day. Temperature rose to 101.5 F. on the third day, but fell to normal shortly after. On tenth day of life the baby showed retraction of neck, nystagmus and twitching of the extremities. During the next three days these symptoms diminished considerably but the general condition was very poor.



On the thirteenth day after birth an abscess of left cheek was opened. At this time a discoloration of finger tips of left hand and toes of left foot was noted. This suggested beginning gangrene. Child grew gradually worse and died December 5, 2 a. m. Body placed in ice-box until time of autopsy. Clinical impression: Septicemia; meningitis; gangrene.

December 6, 11 a. m. Autopsy performed thirty-three hours post-mortem. Blood in heart and large vessels was quite fluid; no clots. This blood clotted readily in contact with the cut tissues. Some of it collected in a test-tube showed no clot at the end of two hours but a firm normal clot at the end of twenty hours.

*Necropsy.*—Abstract of Protocol 3823.

*Anatomical Diagnosis:* Multiple thromboses involving umbilical vein, hypogastric arteries and external iliaes, ductus Botalli, aorta and portal vein; aneurysm of ductus Botalli; subdural hemorrhages posterior to cervical cord; slight internal hydrocephalus; pseudo-infarct of liver; fatty degeneration of viscera; atelectasis; bronchopneumonia.

Body is that of an emaciated, colored baby two weeks old, 50 cm. in length. Rigor mortis is present. The stump of the umbilical cord appears normal. There is an abscess on the left cheek from which exudes bloody pus on pressure. The left foot and ankle are of a deep purple color. On section abdomen appears normal. The peritoneal surfaces are smooth and glistening. The mesenteric lymph-glands are injected and seem slightly enlarged and in places hemorrhagic. Thymus is normal. The pericardial sac contains a normal amount of fluid. The surface is smooth and glistening. The lung surface shows many deeply injected areas varying from 1 to 2 cm. in diameter. On section they are air-containing for the most part, but a few reddish, slightly elevated areas are to be found.

The spleen is rather soft and grayish in color. The Malpighian corpuscles can be seen as minute grayish dots. Stomach and duodenum are normal. The bile ducts are patent.

*Liver:* On the anterior surface of the left and the middle portion of the right lobe is an irregularly outlined area measuring  $4\frac{1}{2} \times 5$  cm. which is deep purple in color. On section the purple area above described is definitely outlined and contrasts sharply with the adjacent tissue. It is of a reddish purple color with indistinct lobulation. The parenchyma elsewhere has a yellowish, opaque cast. The portal vein at the hilus and extending into the substance of the liver is occluded by a thrombus.

The intestines are injected. On opening the aorta a thrombus is found beginning 1 cm. above the bifurcation and extending down into both iliaes, on the left to the opening of the epigastric artery; on the right about 1 cm. below the opening of the right hypogastric artery.

The brain is found to be edematous and the vessels injected, and an excess of fluid flowed from the ventricles. The pia is otherwise normal. On section the lateral ventricles are somewhat dilated. On the posterior surface of the cervical cord is found a clot which covered an area 3 cm. in length. The clot appears recent. Other organs need not be described.

*Microscopical Notes.*—The vessels in some instances show thrombi which are partly organized. Lungs show some interstitial pneumonia somewhat like that seen in congenital syphilis. The alveoli contain an albuminous exudate and many alveolar cells but no fibrin. Some sections show extreme atelectasis. The capillaries of the liver are free from blood islands and there is no evidence of syphilis. Each lobule shows a central fatty degeneration involving about half the parenchyma. Pancreas and other viscera are normal. There is no evidence of syphilis.

This case, although incomplete, evidently presented an excess of anti-thrombin in the blood at time death. There may have been a low prothrombin fraction, but the great acceleration of coagulation of the

blood in contact with the cut tissues speaks against this. We may assume with some certainty that this case showed an upset of the antithrombin-prothrombin balance, and this is of interest chiefly because of its association with extensive vascular thrombosis. This infant probably was suffering from a general infection and the antithrombin excess may be classed with that found in severe septicemia and reported previously (Whipple<sup>1</sup>).

In this instance we believe the excess of antithrombin is in part dependent on extensive thrombus formation. Under such conditions the production of *thrombin* must be considerable, and its escape into the blood-stream inevitable. The introduction of thrombin into the blood-stream causes a transient rise of antithrombin as can be studied in the dog (Howell<sup>4</sup>). When a thrombus is developing slowly in the blood-stream we may assume a steady escape of thrombin and more or less elevation of the antithrombin curve. It may not be far from the truth to think of this antithrombin wave as a process tending to delimit the growth of any thrombus. We may consider this antithrombin reaction under these conditions as protective and tending to stop thrombus development. It will be of interest to follow this antithrombin curve in patients where there is some certainty of a progressive thrombosis or even an endocarditis of the malignant type. It is more than likely that there may appear periods of low antithrombin blood content which would favor the start or growth of a thrombus.

#### APLASTIC ANEMIA, PURPURA, BLEEDING, ANTITHROMBIN

CASE 5.—Rykowski. Autopsy No. 3724. White male, aged 16. Admission April 3, 1912. Death May 6. Patient complained of faintness.

Present illness began nine weeks before death with epistaxis and bleeding of gums. Definite yellow color in skin noted one week before this.

April 3. Red blood-cells 796,000, white blood-cells 2,200. Hb. 15 per cent. Differential count showed small mononuclear leukocytes 83 per cent. Polymorphonuclear leukocytes 14 per cent. Myelocytes  $2\frac{1}{2}$  per cent. Blood clotted in five minutes, but the bleeding time was over twenty minutes. Examination of the eyes showed a definite retinitis with extensive hemorrhage. Sclerae pale and white.

April 10. *Strongyloides stercoralis* found in considerable number in stools. Wassermann negative.

April 16. Bleeding time very much prolonged and gums ooze blood constantly. Hemoglobin has fallen to 10 per cent.

April 17 patient was given an *indirect transfusion* of 500 c.c. defibrinated blood obtained from brother. Shortly after it his temperature rose to 105.6 F. accompanied by a chill.

April 18. Purpura noted in skin and mucous membranes of tongue. Epistaxis started and continued in spite of epinephrin.

April 19. Epistaxis with slow oozing continued. Blood culture and Widal both negative. Hb. 22 per cent. Red blood-cells 1,224,000. W. B. C. 1,280. A few blood platelets were noted.

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4. Personal communication.

April 21. Hb. 17 per cent.

April 27, Hb. 12 per cent. Bleeding continued.

May 3, Hb. 9 per cent. Epistaxis and oozing continued.

May 5, respirations slow and deep. Fresh patches of purpura noted. Urine free from bile, urobilin and albumin. Temperature continued uniformly between 99 and 100.1 F. during the previous few days.

May 6, 12:05 p. m., death. Blood aspirated from heart directly into oxalate at 1:30 p. m.

*Anatomical Diagnosis.*—Hemorrhage in the subcutaneous tissues, pericardium, submucous tissues of the trachea, stomach, intestine and lungs; pericardial effusion; marked anemia; fatty degeneration of heart muscle; tuberculous bronchial lymphadenitis; chronic pleural adhesions; rectal polyps; adenoma of liver; bronchopneumonia with necrosis; central liver necrosis.

*Necropsy.*—Body is that of a very well nourished, white boy, 152 cm. long. The skin is waxy in appearance. The mucous membranes are extremely pale. There are a few small, purpuric spots over the right side of the forehead and elsewhere over the face. There is an excoriation on the left side of the nose and one on the upper lip. A few small ecchymoses over the arm and fading ones elsewhere on the body. These are not conspicuous below the waist except for a rather large conglomerate area on the right leg. Rigor mortis is well marked. The abdominal viscera are normally disposed and serous surface normal. The liver and the rest of the organs are extremely pale. The mesenteric lymph-nodes are large and firm. Sections, yellowish-gray in color, homogeneous in consistence. The thymus is fairly prominent and extends down over the upper part of the pericardium. It contains a great deal of fat. The pericardial surfaces both parietal and visceral are covered with hemorrhages of various sizes from that of a pin-head up to several millimeters in diameter, flame shaped or streaked.

Liver measures 24 x 15 x 7 cm., weighs 1.200 gm. Gall-bladder contains thick, viscid, black bile. The edge is fairly rounded. The color is pale. On section the lobulation is indistinct but regular.

Bone-marrow is firm and yellowish in color, made up almost entirely of fat. There are only a few red specks representing clumps of bone-marrow cells.

*Microscopical Sections.*—Lungs: Areas of bronchopneumonia in which colonies of bacteria are conspicuous. There is much cell necrosis in these areas and fibrin threads are very numerous. Evidently the cell necrosis freed sufficient thromboplastin to neutralize the excess of antithrombin in the blood. One of the large vessels in an area of necrosis contains a small clot in which the fibrin is confined to the margin of the vessel.

Spleen: Malpighian bodies normal. Pulp contains little blood and the venules show slight thickening of their walls. No pigment. Lymph-nodes are negative.

Bone-marrow made up of fat cells. There are two or three clumps of normal marrow cells numbering six to twelve—complete aplasia.

Duodenum shows evidence of old hemorrhage into the villi, where are found numerous phagocytes containing yellow granular pigment.

Liver: The cells show slight increase in the fine, yellow, intracellular pigment. There is a central necrosis involving perhaps one-tenth of the parenchyma, associated obviously with the necrotizing bronchopneumonia. Other organs normal.

*Blood Examination.*—May 6, 12:05 p. m., death. Blood drawn from heart into oxalate at 1:30 p. m. Blood drawn into a clean bottle (no oxalate) started to clot in forty-five minutes, filmy clots forming on the side of the container. After wiping out with a glass rod more clots formed in one hour. Incomplete coagulation in six hours. After twenty hours the clot was tough and firm, no more forming after this.

Some of this blood (not oxalated) was centrifugalized and this procedure and subsequent handling was associated with acceleration of clotting. The anemia was profound and 50 c.c. of blood after centrifugalization yielded only about 1 c.c. of red cells.

TABLE 7.—CASE 5. COAGULATION TIME OF BLOOD AFTER DEATH

Plasma H c.c.	Plasma D c.c.	Calcium Drops	Coagulation Time Minutes	
			Start	End
..	1	5	—	5
1	..	5	15	17
1	1	10	9	—
2	1	15	14	—
3	1	20	11	—

Plasma D was from a normal dog. The coagulation of all mixtures of plasma H was in a fractional manner as described for the whole blood, although very much less marked. (Table 7.) After twenty-four hours the same plasmas were tested with some fresh spleen extract.

TABLE 8.—CASE 5. COAGULATION TIME WITH SPLEEN EXTRACT

Plasma H, c.c.	Plasma D, c.c.	Spleen Extract Drops	Calcium Drops	Coagulation Time, Minutes	
				Start	End
1	..	..	5	30	..
..	1	..	5	..	6
1	..	6	5	..	2
..	1	6	5	..	1

Tables 7 and 8 and the observation on the whole blood give conclusive evidence of an excess of antithrombin. There is no lack of prothrombin, as the spleen extract clots the human plasma at once, due simply to a neutralization of the antithrombin, which is the only abnormal factor in the equation.

It is of interest to note the effect of an indirect transfusion which was followed by a febrile reaction and doubled the patient's hemoglobin. The bleeding continued and evidently the antithrombin excess was in no great degree influenced by this procedure. On theoretical grounds it does not seem justifiable to introduce defibrinated blood which is rich in thrombin into a circulation in which antithrombin is in excess. There is every reason to suppose this procedure would be followed by an antithrombin wave which might make matters even worse. The transfusion if attempted should be direct.



LEUKEMA, PURPURA, BLEEDING, ANTITHROMBIN

CASE 6.—Shema. Medical No. 31151. White male, aged 26. Admission July 7. Patient was in a desperate condition due to repeated hemorrhage. Patient had suffered from severe hemorrhages from the nose during the past three weeks. The nasal passages had been packed but the bleeding was not completely checked. Gums had been oozing constantly up till the last day of life. Patient was dyspneic and very weak. There was no history of any previous attacks of hemorrhage, and family history was normal. Patient's past history was negative except for an attack of gonorrhea about five years previously, associated with some joint complications.

*Examination.*—The patient is very pale and thin. Respirations were of air hunger type. Purpura everywhere over trunk and limbs. The patches were of all sizes from pin-head specks up to spots 1 cm. in diameter, both old and recent. There was edema of feet and ankles. No icterus. The spleen was enlarged. Red blood-cells 1,092,000. W. B. C. 75,300. Hb. less than 10 per cent. Blood smears examined by Dr. Guthrie gave evidence favoring the diagnosis of chronic myelogenous leukemia. There were numerous immature polymorphonuclear leukocytes and myelocytes. Eosinophils were numerous. Nucleated reds very abundant.

Death occurred at 12 m. July 7. No autopsy. Blood aspirated from heart directly into oxalate at 3:30 p. m. Blood also drawn directly into a clean bottle with no oxalate and centrifugalized. During this process a flabby clot formed at 3:50 p. m. This small clot was removed and other secondary clots reformed and were removed at intervals during the next two hours. Small clots formed in this same plasma over night, completing the coagulation, and no subsequent clots reformed after this time. There was no autolysis of the blood clot.

Plasma and serum C from a normal cat. Spleen extract (dilute) from same cat sacrificed at time of experiment. (Table 9.)

TABLE 9.—CASE 6. COAGULATION TIME OF BLOOD AFTER DEATH

Plasma H, c.c.	Plasma C, c.c.	Serum C, c.c.	Spleen Extract Drops	Calcium Drops	Coagulation Time, Minutes	
					Start	End
1	.....	.....	.....	3	17	*25-60+
.....	1	.....	.....	2	4	5
1	1	.....	.....	5	4½	7
2	1	.....	.....	7	6	*8+
3	1	.....	.....	9	7	*10+
1	.....	1	.....	2	3	10
1	.....	.....	15	2	..	3
1	.....	.....	5	2	.....	11
.....	1	.....	.....	2	.....	4
.....	1	.....	5	2	.....	11½
.....	1	1	.....	2	.....	11½
.....	1	.....	15	2	.....	¾

\* Coagulation proceeded slowly in fractions after clots were wiped out by glass rod at intervals.

Table 9 shows clearly that the human blood contained an excess of antithrombin and otherwise was normal. The cat serum contained a good deal of thromboplastin and accelerated coagulation of normal as well as human plasma. The human plasma caused definite delay in

coagulation of normal cat's plasma. The fibrinogen was low, but not under 0.1 gm. per 100 c.c.

The two preceding cases furnish evidence that the purpuras and various hemorrhagic symptoms often associated with blood diseases, especially leukemia, are due to an excess of antithrombin. The case of aplastic anemia with inactive bone marrow almost completely excludes the bone marrow as a factor in this antithrombin upset.

It is unfortunate that the platelets were not enumerated in these two cases. It is possible that they were diminished in the first case of aplastic anemia, but very unlikely in the second case (Shema) of chronic leukemia. The platelets are usually increased in number in myelogenous leukemia.

Duke<sup>5</sup> has recently called attention to certain cases of purpura and bleeding in which the platelets are greatly diminished. He believes this to be the sole cause of the tendency to hemorrhage, but it does not seem that this point has been established beyond reasonable doubt, as he has not followed the elements of blood coagulation. The bleeding time in chloroform poisoning may be hours and the coagulation time normal. The platelets are normal or increased, and the essential lesion is a great drop in fibrinogen, rendering the clots too flabby to check hemorrhage.

In the cases reported by him, it is possible that an excess of antithrombin was present. Blood drawn through the tissues (ear puncture) is of no value for the determination of antithrombin (when negative) as the tissue juices may have neutralized all the excess of antithrombin. There may be a sufficient excess of antithrombin to overcome the thromboplastin of the tissues and favor prolonged oozing from cuts and punctures (delayed bleeding time). One can imagine an excess of antithrombin capable of delaying the coagulation of the blood on contact with injured tissues (ear puncture) and so favoring bleeding, yet this excess might be used up by this very contact with the cut tissues, and the blood escaping give a coagulation time of seven to eight minutes. Duke's suggestion that the platelet thrombi normally forming at the ends of the cut vessels are a great aid in stopping hemorrhage is perfectly logical and probably true. There may well be other factors concerned in the diseases which he has studied, and these should be controlled, as the platelet theory is more or less a mechanical explanation.

It is conceivable that the thromboplastic content of various tissues may vary widely under different conditions and may be concerned in promoting or checking hemorrhage when the normal factors of blood coagulation are upset. It is not difficult to imagine a drop in thromboplastin tissue content when there has been a long period of antithrombin excess in the blood, and it is possible that this may be an important

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5. Duke, W. W.: *THE ARCHIVES INT. MED.*, 1912, x, 445.

factor. The methods available at present give unsatisfactory results in attempting estimates of the thromboplastin content of various organs and tissues.

There is a tendency among certain writers to attribute the symptoms of hemorrhagic disease, purpura, bleeding from the mucous membranes, etc., to a temporary increase in fragility or permeability of the capillary vessels. This seems to us to be merely avoiding the issue and ignoring well established facts, which alone are sufficient to explain all the symptoms. It will be admitted that bleeding from the gums, mucous membranes, skin punctures, etc., can be produced experimentally in a variety of ways. We may produce it experimentally by chloroform poisoning, which acts by decreasing the fibrinogen almost to zero in extreme cases, and by peptone injections under favorable conditions where an excess of antithrombin is the essential feature. The bleeding will appear within a few hours after this abnormal condition of the blood appears and vanish as the blood returns toward its normal equilibrium. Surely it is asking much of one's imagination to postulate a variation in fragility of the vessel walls during this short space of time. It will be noted, too, that the bleeding occurs around the teeth where there is a constant inflammation and tissue injury due to trauma and bacteria. The mucous membranes are delicate and subjected to constant trauma which we must imagine cause trivial injuries to the surface and capillaries which are so close to the surface. It will be recalled that purpura is always most marked on surfaces exposed to trauma. It is not too much to suppose that trifling capillary injury is being constantly repaired under normal conditions. Given an abnormality of coagulation, even the most trivial injury may permit of escape of blood into the tissues, or from the surface of the mucous membranes.

#### MELENA, NEONATORUM—PROTHROMBIN ABSENT

CASE 7.—Baby Chapman, age 2 weeks. Birth May 26, 1912. Death June 10, 2 a. m.

*Clinical History.*—Mother, aged 25, has had two children and one miscarriage. The last child born about a year ago is healthy. Mother gave a positive Wassermann reaction.

May 26. Spontaneous delivery with no difficulty. Placenta normal; no evidence of syphilis. Child measured 47 cm. and weighed 2,800 gm. at birth. The weight of the child diminished as usual and then remained constant with no upward tendency. During the last week weight fluctuated between 2,500 and 2,450 gm. Temperature was normal.

June 4. Retraction of foreskin, which was associated with a good deal of bleeding.

June 5. Continuous oozing from foreskin in spite of all efforts to check bleeding.

June 6. Hemorrhage continued in spite of clamps, various styptics and actual cautery. Human serum, 3 c.c., given subcutaneously. Icterus of the conjunctivae noted.

June 7. No bleeding. Condition much better, given calcium lactate.

June 8. Human serum, 2.5 c.c., given subcutaneously; slight bleeding noted from the cord and little blood in vomitus. No blood in stools.

June 9, 9 p. m. Considerable hemorrhage from the umbilicus, followed by great loss of strength and death June 10 at 2 a. m.

*Clinical Diagnosis.*—Hemorrhagic disease of new-born: syphilis.

*Autopsy* June 10, 10 a. m. No. 3735.

*Anatomical Diagnosis.*—Hemorrhagic disease; absence of blood clots in vascular system; general icterus; subcutaneous and pulmonary hemorrhages; acute splenic tumor; anemia; bronchopneumonia; absence of prothrombin.

Body is that of a well-developed normal appearing negro male infant, 47 cm. in length. The body is cool and rigor mortis is present. Jaundice is well marked in the mucous membranes, conjunctivae and subcutaneous tissues. Thorax opened and blood aspirated from the heart *in situ*; about 30 c.c. of blood obtained. The blood is quite fluid, and there is no evidence of any clot formation in any part of the vascular system. Thorax normal, except for some small areas of purpura in the subcutaneous tissue over the thorax. There are ecchymoses in the thymus and indefinite purplish splotches in the lungs. Thymus is of normal size, very pale, lemon-yellow color and translucent, normal on section.

Heart is of normal size, firmly contracted. The intima and valves throughout are quite normal. Heart muscle is of a pale brownish red color and uniform on section.

Lungs are voluminous and pale lemon color, downy feeling anteriorly, rather heavy and moist posteriorly. The bronchi contain slimy mucus and some vomitus. The mucosa is pale everywhere. The vessels at the hilum are normal and contain no clots. Section shows pale, uniform upper and anterior portion, while the posterior portions show a mottled appearance due to indefinite splotches and specks of bright purple color, looking more like tiny areas of hemorrhage than like areas of consolidation.

Spleen weighs about 6 gm. It is about double the size of the normal spleen of an infant of this size. It is soft; the edges are rounded. The capsule is thin and delicate. Section shows a rather velvety and deep purplish pulp having a chocolate tinge. The Malpighian bodies are small and cleanly outlined. The pulp scrapes off easily and obscures the trabeculae.

Liver is normal size and definitely jaundiced, having an olive-green color with a slight yellowish tint. The lobulation is regular. The cut section is uniform and rather translucent. The gall-bladder contains thin fluid bile and is not particularly distended. No obstruction to the outflow of bile can be made out. Pancreas is of normal size, very pale, milky-white and uniform in appearance.

Mesenteric glands are definitely enlarged and rather conspicuous in the mesentery where the fat has undergone some regression. The lymph-nodes in other parts of the body are rather conspicuous but uniformly pale and translucent on section.

Stomach contains curds and milky fluid. The mucosa is pale. There is no evidence of any hemorrhage. The mucous membrane is intact throughout the intestinal tract wherever examined. The small and large intestine show no evidence of any blood escaping from the mucosa. Intestines are decidedly pale, and except for a certain amount of distention with gas, perfectly normal in gross. Adrenals are rather smaller than normal, about one-half the size of a normal child, but uniform in appearance, with a regular cortex and medulla.

Kidneys are normal. Bladder and prostate are normal. Aorta is perfectly elastic and normal throughout.

Bones: The line of ossification at the lower end of the femur is slightly wider than normal but straight, regular and sharply outlined. The bone-marrow has a currant red and juicy appearance. Brain could not be examined.

*Microscopical Notes.*—Thymus is normal, not of regressive type. Mononuclear eosinophils are numerous.



Lungs show lesions suggestive of congenital syphilis. The septa are thick and the stroma rich in mononuclear cells. In places are patches of desquamative pneumonia; no fibrin is found in any section. The blood-vessels show thickened walls and the capillaries are relatively inconspicuous in the walls of the alveoli.

Spleen shows normal Malpighian corpuscles. The pulp is full of blood and the sinuses engorged. Phagocytosis by endothelial cells is rare. Nucleated red cells are numerous. Few eosinophils. Mesenteric glands are uniform. Sinuses dilated with endothelial cells, many of which are actively phagocytic, some including as many as six mononuclear cells. Kidney, adrenal and prostate are normal.

The liver cells are normal except for conspicuous bile canaliculi filled with greenish colloid. Between the columns of liver cells are a few nests of mononuclear cells like those seen in congenital syphilis. Levaditi stains in all organs show no spirochetes.

*Blood Examination.*—Blood taken from right auricle at 10:30 a. m. There were no clots to be found anywhere in the vascular system after the most careful search.

1. Blood containing no oxalate (about 10 c.c.) taken from heart. The corpuscles settled by gravity giving material used in Table 10 (Plasma H, non-oxalated). There was no clotting after a period of forty-eight hours.

2. Blood 20 c.c. added to 2 c.c. sodium oxalate (1 per cent.) and centrifugalized, giving a slightly turbid orange-yellow plasma whose froth was definitely greenish from the presence of bile pigments. The calcium oxalate precipitate was more abundant than usual after centrifugalization. Plasma H—oxalated—used.

3. Spleen Extract H, made by grinding the spleen pulp with sand and two volumes of Ringer's solution followed by filtration.

4. Spleen extract D, fresh extract of same type from dog.

5. Plasma D, and 6, Serum D, both fresh plasma and serum from normal dog.

TABLE 10.—CASE 7.—COAGULATION TIME OF BLOOD AFTER DEATH

Plasma H, Oxalate, c.c.	Plasma H, No Ox. c.c.	Plasma D, c.c.	Serum D c.c.	Calcium Drops	Coagulation Time Minutes
..	1	..	1	..	9
..	1	..	2	..	9
..	..	1	..	5	7
1	..	..	..	5	*
1	..	1	..	7	3
2	..	1	..	12	4
2	..	1	..	12	4
..	..	1	†	10	5

\* = No clot in forty-eight hours. † = 1 c.c. Ringer's solution.

1. Dog plasma (1 c.c.) + spleen extract H, 7 drops + calcium. 5 drops = clot in 1½ minutes.

2. Plasma H., no oxalate (1 c.c.) + spleen extract H, 9 drops = no clot in 24 hours; at this time clotted by adding serum D.

3. Plasma H., oxalated (1 c.c.) + spleen extract D, 5 drops = no clot in 24 hours; then clotted by serum D.

4. Plasma H., oxalated (1 c.c.) + spleen extract D, 4 drops = no clot in 24 hours; then clotted by serum D.

5. Plasma H., oxalated (1 c.c.) + serum D (1 c.c.) = clot formed in 12 minutes.

6. Plasma H., oxalated (1 c.c.) + pure thrombin, 7 drops = clot; starts in 6 minutes; complete in 10 minutes.

7. Plasma H., no oxalate ( $\frac{1}{2}$  c.c.) + pure thrombin, 7 drops = firm clot in 13 minutes.

The data above make it absolutely certain that in this case of melena neonatorum the only abnormality was the absence of *prothrombin*. The plasma is readily clotted by fresh serum or pure thrombin which later was obtained through the kindness of Dr. Howell and prepared after his method described in a recent publication. This plasma does not delay the coagulation of normal plasma showing an absence of any anti-thrombin excess. Fibrinogen and calcium are present at least in normal amounts. The spleen extract from this case contains an abundance of thromboplastin. There is clinical evidence of improvement following treatment by human serum which, of course, furnishes the missing factor of coagulation, but in this case the improvement was transient.

We have reported a similar case in an earlier communication, in which the evidence pointed pretty conclusively to a total absence of prothrombin. This case strengthens the argument that this group of cases is quite clean cut with constant blood-findings. There was conclusive proof of fibrin formation in the lung of this case previously reported, but this coagulum had been formed several days previous to the onset of hemorrhagic tendencies and death. Also this first case gave no evidence of any organic or infectious disease and no hint of the source of prothrombin formation.

The case reported here shows evidence of the syphilitic virus although no spirochetes can be demonstrated and the placenta was normal. It is possible that syphilis was important in the etiology of this case, but study of the organs gives no clue to the origin of prothrombin.

#### CONCLUSIONS

The antithrombin-prothrombin balance in the blood is in delicate equilibrium but under normal conditions there are strong factors which can preserve this balance. Under experimental conditions this may necessitate rapid neutralization of antithrombin excess; again a rapid production of fresh antithrombin or prothrombin. There are wide margins of safety in the normal animal.

This antithrombin-prothrombin balance may be temporarily or permanently upset under disease conditions as reported in the preceding cases. The *prothrombin* factor is rarely involved, but it may drop to zero (Case 7) or to a low level (Case 1) which will be associated with hemorrhagic symptoms.

The *antithrombin* factor is frequently involved and if this element is much increased above normal there will be a tendency toward hemorrhage, purpura, etc., depending in part on the duration of the change, but especially on the amount of antithrombin excess.

It may be assumed that during these wide fluctuations of *antithrombin* it may fall *below* normal, whereupon there would be a tendency to vascular thrombosis. Given a low antithrombin content a minor injury to the intima might initiate a thrombus which would grow rapidly. With the growth of a thrombus there would be the formation of much thrombin which would escape into the blood and stimulate the organ (liver) producing antithrombin. This might result in an antithrombin wave—a period of antithrombin excess (Case 4)—which would check the growth of thrombi and give the endothelium opportunity to cover over the fresh thrombi, thus removing the foci of thrombin formation. The antithrombin wave may fall promptly to normal or persist for weeks, but if its duration is a matter of days there will appear signs of hemorrhagic disease (Case 2).

It seems highly probable that under certain conditions liver injury or disease may be associated with an excess of antithrombin in the blood capable of giving hemorrhagic symptoms (Cases 1 and 2). Also certain substances in the blood (peptone, thrombin) will bring about an overproduction of antithrombin due probably in great part to stimulation of the liver. Under this heading may be grouped cases of septicemia (reported previously), pneumonia (Dochez) endocarditis, miliary tuberculosis (Case 3) generalized vascular thrombosis (Case 4), etc.

True hemorrhagic disease is rarely associated with simple obstructive icterus, contrary to common belief. Delayed coagulation time in jaundice may be influenced favorably by calcium, but such cases do not suffer from bleeding unless there is some other abnormality—for example, an excess of antithrombin. In these rare cases of true hemorrhagic disease associated with profound icterus, we may presuppose some liver disease and an upset in the antithrombin-prothrombin balance (Case 1). Calcium has no effect on this condition.

Disease of the blood-forming organs may present symptoms of hemorrhagic disease (aplastic anemia, Case 5 and leukemia, Case 6) because of an excess of antithrombin in the blood. It is unlikely that the blood-forming tissues are directly concerned in the antithrombin production as this element is much in excess in the case of anemia (Case 5) with complete marrow aplasia. It is possible that the products of blood-cell disintegration may stimulate an overproduction of antithrombin. It will be recalled that purpura and hemorrhagic tendencies are more common in acute leukemias in which the evidences of blood-cell disintegration are most marked. It is probable that the majority of hemorrhagic

cases and purpuras associated with leukemias and anemias belong in the antithrombin group.

The disease called *melenaeonatorum* in many, perhaps all, instances is characterized by a relatively sudden disappearance of prothrombin from the blood. This condition usually develops during the first two weeks of life and is often fatal. The cases react favorably to fresh serum treatment.

*Treatment* of hemorrhagic disease should follow a careful analysis of the blood as harm can be done by faulty treatment. In cases of low or absent prothrombin, it is clear that serum which is rich in this element is indicated and in many cases produces almost miraculous cures. It should be given intravenously if possible. Direct transfusion is of even greater value.

Treatment of antithrombin cases offers great difficulties. Serum treatment offers no help and it may even stimulate a still greater overproduction of antithrombin. Indirect transfusion is open to the same criticism (Case 5) as large amounts of thrombin are introduced. It may be possible to find some safe way to introduce thromboplastin into the blood-stream in the hopes of neutralizing the antithrombin excess. If the means by which the antithrombin is neutralized or used up in the normal body can be found out by various animal experiments, the solution of this problem in treatment of antithrombin cases may be reached. At present direct transfusion seems to offer the greatest hope of permanent benefit.



PRACTICAL STUDIES ON THE SO-CALLED SYPHILIS  
"ANTIGENS," WITH SPECIAL REFERENCE TO  
CHOLESTERINIZED EXTRACTS \*

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PHILADELPHIA

Of most interest and importance in the serum diagnosis of syphilis is the question of "antigens," not only from the standpoint of efficiency and specificity, but also from the possibility of throwing more light on the nature of the reaction.

When Wassermann, Neisser and Bruck originally applied the Bordet-Gengou phenomenon of complement-fixation to the diagnosis of syphilis, the causative organism, *Treponema pallidum*, had not been isolated in pure culture, so they selected the fetal liver of congenital syphilis because rich in the *Treponema*, for making the antigen. Securing well-marked and definite results, they concluded that the specific antigenic properties of the extract were due to the presence of the *T. pallida* and that the reaction was an example of specific union of antigen and antibody. Subsequent investigation indicates that this is partially true, as the antigenic principles of *T. pallida* are extractable in salt solution, and in some cases of syphilis there exists a true antibody which will unite with the true *T. pallida* antigen. But it is probable that if the original investigators had had a pure culture of *T. pallida* for antigen the great practical value of the syphilis reaction would not have been readily realized, as recent work with *T. pallida* antigens shows weaker and more inconstant results and is of less practical value as compared with tissue extracts.

The belief in the specific nature of the "antigen" was early shaken by the investigation of Marie and Levaditi,<sup>1</sup> who showed that an extract of a normal liver could replace an extract of a syphilitic liver in the reaction. This discovery was abundantly confirmed by the subsequent investigations of Porges and Meier,<sup>2</sup> Landsteiner, Müller and Potzl,<sup>3</sup> Levaditi and Yamanouchi,<sup>4</sup> Noguchi and Bronfenbrenner,<sup>5, 6</sup> and others.

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\* Submitted for publication July 16, 1913.

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1. Marie, A., and Levaditi, C.: Ann. de l'Inst. Pasteur. 1907, xxi, 138.

2. Porges, O., and Meier, G.: Berl. klin. Wehnschr., 1908, p. 731.

3. Landsteiner, K., Müller, R., and Potzl, O.: Wien. klin. Wehnschr., 1907, p. 1565.

4. Levaditi, C., and Yamanouchi, T.: Compt. rend. Soc. de biol., 1907, lxiii, 740.

5. Noguchi, H.: Jour. Exper. Med., 1909, xi, 84.

6. Noguchi, H., and Bronfenbrenner, I.: Jour. Exper. Med., 1911, xiii, 43.

with various extracts of various non-syphilitic organs. These investigations also showed that the "antigen" was soluble in alcohol and probably of the nature of a lipid. Hence the original conceptions of the reaction were not tenable, although it is fortunate that a tissue was selected for the original "antigen"; otherwise this reaction, of great diagnostic value and therapeutic guidance, may have been delayed in its development.

As soon as the important rôle of lipoids in this reaction was demonstrated, attempts were made to substitute the complicated tissue extracts with pure solutions of lipoids. Among these, lecithin, sodium taurocholate, glycocholate, oleic acid, oleates, cholesterin, etc., have been tried alone and in combination. It was soon found that many tissue extracts were unsuitable for the reaction because of the presence of impurities which caused non-specific inhibition of hemolysis or were in themselves hemolytic. Noguchi, and later Noguchi and Bronfenbrenner, endeavored to purify extracts by fractional solution and precipitation and succeeded in devising an extract of acetone insoluble lipoids composed largely of lecithin which has given good results. Later, Browning, Cruickshank and Mackenzie<sup>7</sup> found that a mixture of lecithin and cholesterin yielded particularly specific reactions in syphilis. The latest notable advance has been the suggestion of Hans Sacks<sup>8</sup> to combine the alcoholic extracts of normal organs with cholesterin. He has found such preparations to possess properties equal to the best syphilitic extracts. This work has been confirmed by McIntosh and Fildes,<sup>9</sup> who feel that in this combination the requirements of a "standard antigen" have finally been realized. We have studied these antigens with particular care, the results being given in this communication.

It was hoped that with the isolation of the *Treponema pallidum* the question of specific antigen would be solved. Noguchi<sup>10</sup> having accomplished the difficult task of isolation, prepared an antigen by extracting several strains in salt solution and used the antigen in the serum diagnosis of syphilis with the result that a specific antibody was demonstrated, especially in tertiary syphilis, or cases under treatment. Noguchi considered that reactions with specific *T. pallida* antigen would indicate the degree of resistance of the patient as shown by the amount of complement fixed, depending on the quantity of specific antibody present in the patient's serum. The work as continued by Craig and

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7. Browning, C. H., Cruickshank, J., and Mackenzie, J.: Biochem. Ztschr., 1910, xxv, 85; Jour. Path. and Bact., 1910, xiv, 484.

8. Sacks, H.: Berl. klin. Wchnschr., 1911, p. 2066.

9. McIntosh, J., and Fildes, P.: Ztschr. f. Chemotherapie, 1912, i, 79.

10. Noguchi, H.: Jour. Am. Med. Assn., 1912, lviii, 1163.

Nickols<sup>11</sup> and ourselves,<sup>12</sup> shows that reactions with *T. pallida* antigens are generally weaker and more inconstant than reactions with tissue extracts and are of limited value. Craig and Nickols also showed that fixation would occur with antigens of *S. pertenius* (yaws) and *S. microdentium*. This indicates a group reaction, and when these results are compared to similar studies of others and our own on the differentiation of streptococci, diphtheria bacilli and the typhoid-colon group of bacilli by specific complement fixation reactions, it is evident that either the amboceptor produced by a member of the group is not specific for its antigen or that by our present methods we are unable to extract the definite and specific antigenic substance from the organism.

The sum total of investigation indicates, therefore, the presence of two antisubstances in the serum of a syphilitic patient. One is of the nature of a reactionary substance of the body cells, a product of decomposition or an altered body protein ("reagin"), which has as its chief property an affinity for lipoids. This substance is regularly produced by the *Treponema pallidum* and is responsible for the positive complement "fixation" with lipoidal extracts. A similar "reagin" is produced during infections with the spirochete of yaws and the bacillus of leprosy and is responsible for the positive Wassermann reaction found in these diseases. Our work with spirochete antigens indicates also that this "reagin" will yield non-specific reactions of minor degree with the lipoidal substances present in culture media and other alien organisms, as *B. typhosus* and *B. cholera*. The true nature of this "antibody," so well designated by Simon as "lipodophilic," is unknown, although the work of Klausner, Noguchi, Friedmann and others, tend to show that it is of the nature of a globulin.

The second antisubstance is probably the true pallida antibody in the nature of an amboceptor, although as far as has been demonstrated by complement fixation reactions, its presence in syphilitic serums is inconstant, and, as already mentioned, it reacts with a group of spirochetes closely resembling the *Treponema pallidum*.

However, since a lipodophilic "reagin" seems to be such an important and prominent factor in the serum diagnosis of syphilis, because of its affinity for lipoids, it may be that considerable light may be thrown on the specificity and nature of Wassermann's reaction by a closer study of lipoids. As will be shown in this communication, there can be little doubt of the importance of cholesterol in this connection and it is not beyond the realm of possibilities that a particular lipid altered by syphilitic infection, may solve the problem of "specificity."

11. Craig, C. F., and Nickols, H. J.: Jour. Exper. Med., 1912, xvi, 336.

12. Kolmer, J. A., Williams, W. W., and Laubaugh, E. E.: Jour. Med. Research, July, 1913.

## ANTIGEN

From the practical standpoint any extract or preparation to be suitable as "antigen" should answer the following requirements:

1. It should possess a high degree of sensitiveness and specificity for syphilitic serum.
2. It should not give false reactions with normal serums, a proper technic being used.
3. It should be reasonably stable and not difficult of preparation, and different preparations should bear a reasonable relationship to each other in their properties.

The large number of different extracts and combination preparations of the lipoids advocated from time to time bear definite witness to the unsatisfactory state of our knowledge and is largely responsible for the inconstant and varying results of different workers, and demonstrates the need of a "standard antigen."

We have made an extensive comparative study of the usual and better known "antigens," and the object of the present communication is to briefly record our results from a practical standpoint, along with a technic which we are using and which in our experience renders the reaction more reliable and less apt to yield pseudo-reactions, namely, to use several "antigens" with each serum being tested, for results show quite definitely that the lipodophilic reagin or the so-called syphilis "antibody" in different serums has a different affinity for different lipoidal extracts, and that these differences are independent of any changes occurring in the "antigens" themselves. With the serum of active syphilis most any extract will serve to yield a positive reaction, but when the amount of "reagin" is small, as in long-standing or vigorously treated cases, the question of "antigen" and technic becomes one of considerable importance. It is in such cases that we have noted the varying degrees of reaction with different "antigens" of equal efficiency, and it was not uncommon to find a negative reaction with one antigen and positive with the others. In not a few cases dependence on a single extract would have led to false negative reaction with serums of cases where the treatment was being guided by the serum reactions.

## METHOD OF STUDY

Since alcoholic extract of syphilitic liver is the most widely used "antigen," we have compared the properties of other "antigens" to several such extracts which we have used for over two years, and are of proven value. The method of comparative study consisted, therefore, in using from three to ten different extracts with each serum and comparing results. As will be pointed out later, all extracts were titrated before using and only those included in the study as proved satisfactory.



The antish sheep hemolytic system was used, complement and amboceptor being titrated before tests were set up, and one unit employed. There is such a close quantitative relationship between complement and amboceptor that they must be accurately adjusted to avoid the influence of natural antish sheep amboceptor in human serums. The serums were always inactivated by heating to 55 C. for one-half hour and used in doses of 0.1 to 0.2 c.c. Serum, antigen, amboceptor, complement, corpuscle and hemolytic controls were set up each time as is our routine practice. Serum, antigen and complement are incubated one hour; amboceptor and corpuscles added and reincubated for one to two hours, depending on the rapidity of hemolysis in the controls, and then placed over night in the refrigerator at a low temperature, readings being made the following morning.

#### "ANTIGENS" STUDIED

The following extracts were studied and may be divided as follows:

- I. Extracts of syphilitic liver:
  - a. Four alcoholic extracts.
  - b. Two acetone extracts (Kolle and Stiner).
- II. Extracts of normal organs:
  - a. Alcoholic extracts.
    1. Of normal liver.
    2. Of beef heart.
    3. Of guinea-pig heart.
  - b. Acetone extracts.
    1. Of normal liver.
  - c. Two extracts of acetone-insoluble lipoids (Noguchi).
- III. Cholesterinized alcoholic extracts:
  - a. Extracts of normal organs (Sacks).
    1. Of beef heart.
    2. Of human heart.
    3. Of guinea-pig heart.
    4. Of normal liver.
    5. Of acetone-insoluble lipoids.
  - b. Extracts of syphilitic liver.
    1. Alcoholic extracts.
    2. Acetone extracts.

All antigens were titrated for antigenic, anticomplementary and hemolytic doses. The serum of a secondary case of syphilis is used in amount of 0.1 c.c. with ascending doses of antigen, and that dose of antigen just giving complete inhibition of hemolysis recorded as the antigenic dose. In using this antigen, at least two or three times this antigenic dose is employed because the one unit may be insufficient with other serums. The anticomplementary dose must be at least eight to ten times the antigenic unit before the extract is accepted as satisfactory. This margin of safety we consider advisable, especially with the cholesterinized extracts. This rule was followed with all "antigens" so as to secure uniform comparison of the various extracts.

## PREPARATION OF "ANTIGEN"

1. *Alcoholic Extracts of Syphilitic Liver.*—These were prepared of the liver of syphilitic fetuses in which the *Treponema pallidum* was found. Fatty livers were avoided and those of stillborn fetuses are to be preferred. Not infrequently a fetus is secured with many or all of the usual signs of luetic infection and the mother yield a positive Wassermann reaction and yet one fails to find the *Treponema pallidum* by dark field illumination. Under these circumstances we frequently prepare an antigen but discard it unless it shows a high antigenic value and is free of anticomplementary and hemolytic effects to a safe degree. We have also used an imported German alcoholic extract of syphilitic liver, but have not as yet found these extracts superior to a satisfactory preparation of our own. These extracts are prepared as follows:

A portion of liver is weighed, minced, ground with quartz sand and treated with 10 volumes of ninety-six per cent. alcohol. The mixture is shaken mechanically with glass beads for twenty-four hours and extracted in the incubator for ten days; filtered, measured and loss of evaporation made up. This alcoholic extract is then diluted 1:10 with saline solution and titrated for antigenic, anticomplementary and hemolytic doses. If a shaking apparatus is not available extraction in the incubator should be continued for two weeks.

2. *Acetone Extracts of Syphilitic Liver.*—Such extracts have been claimed by Kolle and Stiner<sup>13</sup> to be more sensitive than alcoholic extracts especially with serums from cases of syphilitic infection of the nervous system. These are prepared as follows:

Two grams of dried syphilitic liver are ground with sand and treated with 60 c.c. of chemically pure acetone, incubated over night, shaken mechanically with glass beads for twenty-four hours, placed at room temperature for forty-eight hours, filtered and titrated.

3. *Alcoholic Extract of Normal Organs.*—These were prepared of the muscular portions of beef heart, guinea-pig heart and of a normal liver containing but a small amount of fat, in exactly the same manner as alcoholic extracts of syphilitic liver except that extraction in the incubator is continued two weeks.

4. *Acetone Extract of Normal Liver.*—This was prepared in the same manner as acetone extract of syphilitic liver.

5. *Acetone-Insoluble Lipoid Extract (Noguchi).*—The preparation of this antigen is so well known that it is only for completeness that the technic of preparation is here included.

A mashed paste of muscle of ox-heart is extracted with ten parts of absolute alcohol at 37 C. for four days. It is then filtered through paper and the filtrate collected and brought to dryness by evaporation. The electric fan is not necessary for the filtrate will evaporate in twelve to twenty-four hours if poured into large flat dishes. The residue is then taken up with a sufficient quantity of ether and the turbid ethereal solution is allowed to stand for a few hours in a cool place until cleared. The clear ethereal portion is then carefully decanted into another clean beaker and condensed into a small quantity by evaporating a portion of the ether. The concentrated ethereal solution is now treated with 10 volumes of pure acetone. A light yellow precipitate forms which is allowed to settle and the supernatant fluid decanted off. Dissolve each 0.3 gm. of this substance in 1. c.c. of ether and add 9 c.c. of pure methyl alcohol. It is usual to find that the greater part of the substance goes into solution. This alcoholic solution remains unaltered for a long time and is kept as stock, from which the emulsion for immediate use may be prepared at any time by mixing 1 c.c. with 9 c.c. of saline solution. This solution is then titrated for antigenic, anticomplementary and hemolytic action. It may be necessary to prepare several

13. Kolle, W., and Stiner, O.: Deutsch. med. Wehnschr., 1911, xxxvii, 1739.

such extracts before one is found thoroughly satisfactory. The ox-heart preparations were uniformly more satisfactory than those of the liver. The main drawback of this antigen is the expense of preparation, and many extracts prove unsatisfactory. For this work we have used three extracts of proved value.

6. *Cholesterinized Alcoholic Extract of Normal Organs.*—These were prepared in the same manner as the plain alcoholic extracts with the addition of 0.4 per cent. of Kahlbaum's cholesterin, or 0.4 gm. per each 100 c.c. of the filtrate, this amount having been found by Swift most satisfactory. All of this cholesterin does not usually go into solution and it may be necessary to refilter after vigorous shaking and standing over night. The extracts of human and beef heart have been better in our hands than the extracts of guinea-pig heart, insofar that the latter extract may be too highly anticomplementary. We have attributed this to the presence of fat and the extraction of undesirable derivatives. It is advisable, therefore, to use only the muscular portions. As will be pointed out later, these extracts possess such high antigenic value that they must frequently be highly diluted to appreciate their sensitiveness.

7. *Cholesterinized Extract of Acetone-Insoluble Lipoids in Methyl Alcohol.*—This was prepared by adding 0.4 per cent. cholesterin to a suitable extract of acetone-insoluble lipoids in pure methyl alcohol. Since the plain alcohol soluble acetone-insoluble fraction is composed largely of lecithin, the extract now becomes essentially a mixture of lecithin and cholesterin.

8. *Cholesterinized Extract of Syphilitic Liver.*—Two alcoholic and one acetone extracts of syphilitic liver were prepared according to the methods already given and cholesterinized by adding 0.4 per cent. of pure cholesterin.

#### COMPARISON OF ANTIGEN PROPERTIES

##### *I. Extracts of Syphilitic Liver:*

(a) *Alcoholic Extracts of Syphilitic Liver:* These extracts are probably more widely used than any other and are regarded by many in higher favor than extracts of normal organs, because of the possibility of their containing products of *T. pallidum* itself. On the other hand, there is little doubt that precedent has considerable influence and the impressions of the original explanation of the reaction is largely responsible for a certain hesitancy in depending on other extracts. One fact should be borne clearly in mind, however: that one good extract of a normal organ is better than a number of poor extracts of syphilitic liver or of liver supposedly syphilitic. We have used several good extracts throughout the study, and, as before stated, results are recorded in comparison to these. Occasionally we have used an imported German extract of syphilitic liver for comparison with our extracts, but the results were entirely similar to those obtained with our own preparations.

While in general it is a difficult matter to express percentages of positive reactions in the later stages of syphilis because of the difficulty of obtaining a reliable history or of indefinite symptoms, we have found our alcoholic extracts of syphilitic liver to yield 87.2 per cent. positive reactions in the primary stage; 96.7 per cent. in the secondary and about 90 per cent. in the tertiary stages, a large proportion of our tertiary cases being infections of the nervous system. With cholesterinized extracts

the percentage of positive reactions in the tertiary and latent stages, or after vigorous treatment, is considerably higher.

(b) Acetone Extracts of Syphilitic Liver: Two extracts were prepared and used with alcoholic extracts of syphilitic liver in testing 443 serums and forty-two cerebrospinal fluids. Of this number of tests, 227 yielded positive reactions.

1. In 68.7 per cent. of cases the acetone extracts yielded results equal to those obtained with the alcoholic extracts.

2. In 4.4 per cent. the acetone extracts yielded stronger or better marked reactions.

3. In 18.9 per cent. of cases the reactions with the acetone extracts were weaker than those with the alcoholic extracts.

4. In 15 or 6.6 per cent. of reactions the acetone extracts yielded negative reactions with the following cases, whereas the reactions were positive with the alcoholic extracts. The cases were as follows:

Two cases of primary syphilis.

Two cases of paresis.

One case of tabes dorsalis.

Three cases of hemiplegia with positive histories.

Five cases of tertiary syphilis with gumma formation.

One case with leg ulcers and positive history.

One case of hereditary syphilis.

5. In the following three, or 1.3 per cent. of cases, the acetone extracts yielded positive reactions, whereas these were negative with the alcoholic extracts:

One case of paresis.

Two cases of tertiary syphilis with positive histories.

It will be noted that acetone extracts of syphilitic liver have not been found superior to alcoholic extracts, and in about 25 per cent. of cases yielded reactions which were weaker than those with alcoholic extracts, or clearly negative. In reference to pseudo-reactions with serums of patients with high temperature, lead poisoning, etc., the results were comparable to the alcoholic extracts in every particular. When suitably preserved, these antigens have been found quite stable.

## *II. Extracts of Normal Organs:*

(a) Alcoholic Extract of Normal Liver: One such extract was used, although several were made but discarded, because too highly anticomplementary and too low in antigenic value. This extract was used in testing 140 serums and seven cerebrospinal fluids along with the alcoholic extracts of syphilitic liver. Of these specimens, fifty-six yielded positive results.



1. In 73.2 per cent. of cases the reactions with this extract of normal liver yielded a similar reaction to those with the extracts of syphilitic liver.

2. In no instance did this extract yield a stronger reaction.

3. In 23.2 per cent. of cases the extract gave weaker reactions.

4. In 3.5 per cent. of cases this extract yielded false negative reactions.

As will be noted, there can be little doubt that the extract of normal liver was less efficient than the extract of syphilitic liver. When this extract was cholesterinized its antigenic properties were doubled and in results it was equal to, and in some case proved superior to the alcoholic extracts of syphilitic liver. When compared to results obtained with extracts of beef and guinea-pig heart, it will be noted that the liver is least suitable for the preparation of extracts of normal organs.

(b) *Alcoholic Extracts of Guinea-pig and Beef Heart:* These extracts enjoy considerable favor with many workers and are probably the organs best adapted for preparing alcoholic extracts of normal organs, because the anticomplementary effects are likely to be low and the antigenic value sufficiently high as to render them serviceable. In our experience, extract of human and beef heart are more satisfactory, because pig-heart extracts are not infrequently highly anticomplementary, a fact which may be attributed to the presence of fat and extraction of undesirable derivatives. As will be seen later, when these same extracts are cholesterinized, the antigenic value is so highly increased as to render them even more delicate than the extracts of syphilitic liver.

Plain alcoholic extracts of pig and beef heart were used in testing 129 serums and ten cerebrospinal fluids, along with the usual extracts of syphilitic liver. Positive reactions were secured with fifty-two of these specimens. Suitable extracts of pig and beef heart are closely similar in antigenic properties.

1. In 71.1 per cent. of cases the reactions were equal to those obtained with the "antigen" of syphilitic liver.

2. In 1.9 per cent. of cases the reactions were stronger.

3. In 13.4 per cent. the reactions were weaker.

4. In the following three cases these extracts gave positive reactions, whereas with the alcoholic extracts of syphilitic liver the results were negative:

One case of lead poisoning with a positive history of lues.

One case of tabes dorsalis.

One case of tertiary syphilis with positive history.

5. In the following four cases these extracts yielded negative reactions with positive results with the alcoholic extract of syphilitic liver:

One case of hemiplegia, history positive.

One case of paresis.

One case of hereditary syphilis.

One case of Jacksonian epilepsy. History and autopsy not obtainable.

(c) Acetone Extract of Normal Liver: One suitable extract was used along with acetone and alcoholic extracts of syphilitic liver in testing twenty-four serums. The results were similar to those of the acetone extracts of syphilitic liver (already given), except that in 12.5 per cent. of cases the reactions were weaker.

(d) Acetone-Insoluble Lipoids (Noguchi): Three satisfactory extracts along with alcoholic extracts of syphilitic liver were used in testing 315 serums and forty-five cerebrospinal fluids, with the following results:

1. In 73.0 per cent. of cases the results were equal.

2. In 10.8 per cent. the reactions were stronger.

3. In 9.7 per cent. the reactions were weaker.

4. In the following five, or 3.2 per cent. of cases, the reactions were positive, whereas with the alcoholic extracts of syphilitic liver the results were negative:

One case of paresis.

One case of tabes dorsalis.

Three cases of secondary syphilis.

5. In the following three, or 1.8 per cent., of cases the reactions were negative, whereas with the alcoholic extracts of syphilitic liver positive reactions were secured:

One case of hereditary syphilis.

One case of paresis.

One case of maternal syphilis.

It must be remembered in this connection that all serums were used inactivated and with an antishoop hemolytic system. Therefore, these were not conducted by the method of Noguchi. We consider a satisfactory extract of acetone-insoluble lipoids as ranking next in efficiency to cholesterinized alcoholic extracts among the "antigens" prepared of normal tissues.

### *III. Cholesterinized Alcoholic Extracts:*

The addition of cholesterol to extracts of normal and syphilitic organs increases the antigenic properties of the "antigen" to a marked degree. The use of such extracts renders the Wassermann reaction even more sensitive, so much so that careful investigation is necessary for ascertaining the probability of false reactions with normal serums. We have found this to occur in a certain proportion of cases, and therefore these extracts, while marking a distinct advance in this field, must be used with care and caution.

The method of preparing these extracts is very simple and has already been given. The addition of 0.4 gm. of pure cholesterin to each 100 c.c. of a filtered alcoholic extract of an organ, preferably human, pig or beef heart. Fat should be avoided.

In this manner we have prepared and studied cholesterinized alcoholic extracts of beef, human and pig heart and human liver. An extract of acetone-insoluble lipoids was cholesterinized and compared to a plain extract; also alcoholic and an acetone extract of syphilitic liver were cholesterinized and compared to plain extracts.

(a) Influence of Cholesterin on Antigen Properties of Extracts: The influence of cholesterin on the antigenic and anticomplementary properties of the extracts was then studied by titration, plain extracts being tested at the same time and in the same manner.

*Method.*—The antigenic titration consisted briefly in diluting the extracts with normal saline solution and placing increasing doses in a series of six test-tubes as follows: 0.01 c.c., 0.05 c.c., 0.1 c.c., 0.2 c.c., 0.25 cc., and 0.3 c.c. One unit of complement and 0.1 c.c. of inactivated serum from a case of secondary syphilis were added to each tube and tubes incubated for an hour, when 1. c.c. of sheep corpuscles and one unit of amboceptor were added. Tubes are reincubated for one to two hours and then placed in the refrigerator over night. Readings are made and recorded according to the amount of inhibition of hemolysis as indicated by the signs, ++++ (100 per cent. inhibition); +++ (75 per cent. inhibition); ++ (50 per cent. inhibition); + (25 per cent. inhibition); ± (less than 25 per cent. inhibition); — (completely hemolyzed). That dose of antigen giving complete or 100 per cent. inhibition of hemolysis is recorded as the antigenic unit. In conducting Wassermann reactions at least two or three times this dose is used, provided that this amount is no more than one-fourth the anticomplementary dose. One may also estimate the antigenic value by determining the number of units of complement "fixed." The anticomplementary titration is conducted in a similar manner by adding 0.1 c.c., 0.2 c.c., 0.4 c.c., 0.8 c.c., 1.0 c.c., and 2.0 c.c. of the diluted "antigen" to a series of tubes, adding a unit of complement and sufficient salt solution. We prefer to incubate these tubes one hour along with the antigenic titration so that the complement is subject to the same deterioration in the incubator. At the end of an hour a unit of amboceptor and corpuscles are added and tubes returned to the incubator for one to two hours, after which readings may be made at once, or after tubes have been placed in the refrigerator over night. That dose of "antigen" just beginning to inhibit hemolysis is recorded as the anticomplementary dose. As is customary, serum, complement, amboceptor and corpuscle controls are set up.

In Table 1 are given some of the results of such titrations of cholesterinized and plain extracts made from time to time for comparison.

The addition of 0.4 per cent. of cholesterin, therefore, increases the antigenic value of an extract at least two to four-fold and does not usually alter the anticomplementary dose. It will also be noted that 0.4 per cent. cholesterin in absolute alcohol, the same proportions as are used in cholesterinizing the extracts, has an antigenic value which may be estimated at about three to six times weaker than cholesterinized tissue extracts. We have not found cholesterinized extracts more stable than plain extracts,

and they must therefore be guarded by frequent titrations and controls against anticomplementary action.

(b) Cholesterinized Extracts and Normal Serums: After using several cholesterinized, along with a number of plain extracts in conducting Wassermann reactions, we soon found that with a number of serums inhibition of hemolysis occurred only with cholesterinized extracts. In the larger number of these instances there was either a history of lues or the clinical condition was such as strongly to indicate this infection. But in other serums where these extracts gave a small amount of inhibi-

TABLE 1.—ANTIGENIC AND ANTICOMPLEMENTARY VALUES OF PLAIN AND CHOLESTERINIZED EXTRACTS

Extracts	Plain Extracts		Same Extracts Cholesterinized	
	Antigenic Unit, c.c.	Anticomplementary Unit, c.c.	Antigenic Unit, c.c.	Anticomplementary Unit, c.c.
Alcoholic, of syphilitic liver, diluted 1:10, No. 1.....	0.1	1.0	0.05	1.0
Alcoholic, of syphilitic liver, diluted 1:10, No. 2.....	0.2	2.0	0.1	1.5
Acetone, of syphilitic liver, diluted 1:10 .....	0.2	0.8	0.1	0.8
Alcoholic, of normal liver, diluted 1:10 .....	0.2	2.0	0.1	2.0
Acetone-insoluble lipoids, diluted 1:20 .....	0.2	1.0	0.15	1.0
Alcoholic, beef heart, diluted 1:10 .....	0.1	2.0	0.05	2.0
Alcoholic, human heart, diluted 1:25 .....	0.1	2.5	0.05	2.5
Alcoholic, pig heart, diluted 1:10 .....	0.1	2.5	0.025	2.5
0.4 per cent. cholesterin in absolute alcohol diluted 1:10 .....	...	...	0.3	1.0

tion the history and clinical condition indicated with a fair degree of accuracy the absence of lues. Since, however, it is a very difficult matter definitely to exclude syphilis in the average hospital case we have tested our own blood and the blood of others, mostly physicians, in whom the possibility of syphilitic infection is definitely ruled out. Twenty such serums were tested as follows: The cholesterinized extracts were used in double the antigenic dose; the serums were inactivated and used in amounts of 0.1 c.c., 0.2 c.c., 0.3 c.c. and 0.4 c.c. The balance of the method was exactly the same as in our routine Wassermann reaction; one titrated unit of amboceptor being used. The results were best marked



with the 0.1 and 0.2 c.c. of serum because the larger doses introduced sufficient natural antisheep amboceptor to cause undue hemolysis. The results of the experiment, therefore, are those found with the 0.2 c.c. dose of serums (Table 2).

It will be noted that the cholesterinized extract of beef heart gave twenty-five or less per cent. inhibition of hemolysis with half of the serums tested, and this extract was certainly not anticomplementary in the dose employed. The remaining extracts yielded pseudo-reactions of about 10 per cent. inhibition of hemolysis with 5 to 10 per cent. normal serums. The sensitiveness of these extracts is also noted in the higher proportion of positive reaction with normal rabbit serum.<sup>14</sup> When using these extracts in amounts of half the anticomplementary dose the per-

TABLE 2.—CHOLESTERINIZED EXTRACTS WITH NORMAL SERUMS

Extracts	Dose Used, c.c.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Cholest. beef heart (1:10).....	0.1	+	—	+	±	—	—	—	±	+	—	—	±	±	±	±	—	—	—	—	+
Cholest. human heart (1:25)...	0.1	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cholest. pig heart (1:10).....	0.1	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—
Cholest. normal liver (1:10)...	0.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	±
Cholest. alcoholic extract syphi- litic liver (1:10) .....	0.1	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	±
Cholest. acetone extract syphi- litic liver (1:10) .....	0.2	±	—	—	—	—	—	—	—	—	—	—	—	—	±	—	—	+	—	—	—
Cholest. acetone insol. lipoids (1:10) .....	0.2	—	—	—	—	—	—	—	—	±	—	—	—	—	—	—	—	+	—	—	—
Plain alcoholic extract syphi- litic liver (control) .....	0.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Serum control .....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

+ = 25 per cent. inhibition.  
— = complete hemolysis.

± = less than 25 per cent. inhibition.

centage of pseudo-reactions is increased. As will be shown later, not a few cases of tertiary lues or cases actively treated with salvarsan and mercury, the quantity of syphilis "reagin" in the serum being small, yielded negative reactions with plain extracts and only 25 per cent. inhibition of hemolysis with the cholesterinized extracts, rendering it difficult to express an opinion in cases more worthy of delicate reactions than those in which the clinical diagnosis is quite evident. We have noted that the syphilis "reagin" apparently varies in different serums in affinity for lipoids in various extracts. For this reason, and especially if employing cholesterinized extracts, it is preferable to use from three to five different extracts with each serum, the total reactions indicating more conclusively the result than one "antigen" alone.

14. Kolmer, J. A., and Casselman, A. J.: Jour. Med. Research, July, 1913.

(c) Cholesterinized Extracts in the Serum Diagnosis of Syphilis: The cholesterinized extracts already mentioned of normal organs and syphilitic liver were used in examining 287 serums and nine cerebro-spinal fluids, plain alcoholic and acetone extracts and acetone-insoluble lipoids being used at the same time for comparison. Of this number of specimens 139 yielded positive reactions.

The results with a cholesterinized alcoholic extract of beef heart, compared to alcoholic extracts of syphilitic liver, are as follows:

1. In 63, or 45.3 per cent. of cases, the reactions were equal to those obtained with the plain alcoholic extract of syphilitic liver.

2. In 41, or 29.4 per cent. of cases, the reactions with the cholesterinized extract were stronger.

3. In 2, or 1.4 per cent. of cases, the reactions with the cholesterinized extract were weaker. Both cases were diagnosed paresis.

4. In no instance occurred a negative reaction with the cholesterinized extract, with a positive result with the control "antigens."

5. In 32, or 23.0 per cent. of cases, the cholesterinized extract yielded a positive result, whereas with the alcoholic extract of syphilitic liver the reactions were negative. These results are worthy of further analysis, especially in view of the fact that we found this extract to give slight inhibition of hemolysis with normal serums.

(a) Of these thirty-two cases, fifteen yielded a weak reaction with the cholesterinized extract, showing about 25 per cent. inhibition of hemolysis (+) as follows:

Two cases of paresis. Positive history in one case.

One case of tabes dorsalis.

One case of tuberculous enteritis. History not obtainable.

One case of optic neuritis.

One case of secondary syphilis.

Two cases of spastic hemiplegia. History not obtainable.

One case of latent lues. Positive history.

One case of lues of three years' duration; thorough treatment with salvarsan and mercury.

One case of recurrent herpes of mouth (patient of Dr. J. F. Schamberg), of one year's duration, resistive to ordinary therapeutic measures. Since Hutchinson has directed attention to the fact that these cases are frequently leptic, Dr. Schamberg is administering antileptic treatment.

One case of hereditary lues.

Three cases of psoriasis. History of infection denied.

One case of recurrent abortion. History of infection denied.

(b) Six cases yielded slightly stronger reactions, about 50 per cent. inhibition (+ +), as follows:

One case of tabes dorsalis. Positive history.

One case of incipient tabes. Positive history.

One case of hemiplegia. Positive history.

Two cases of spastic hemiplegia. History not obtainable.

One case of hereditary lues.

(c) Two cases yielded strongly positive reactions (+ + +), about 75 per cent. inhibition, as follows:

One case of hemiplegia. History not obtainable.

One case diagnosed tuberculosis of lungs. Positive history.

(d) Eight cases yielded absolute positive reactions (+ + + +) or 100 per cent. inhibition of hemolysis. These reactions are most striking and clearly illustrate the tendency of cholesterinized extracts to give strong inhibition of hemolysis in the presence of but small amounts of syphilis "reagin."

One case of secondary syphilis. History positive.

One case of tertiary syphilis. History positive.

One case of hereditary syphilis.

One case of paresis. History not obtainable.

One case of tertiary syphilis (ulcerating gummata).

Two cases of hemiplegia with positive histories.

One case of cirrhosis of the liver. Patient of Dr. Gwyn's. History negative.

Autopsy as reported by Dr. Gwyn showed no evidences of lues.

These results would indicate that any serum showing 50 per cent. or more inhibition of hemolysis may be regarded as luetic. In a number of cases thorough treatment with salvarsan and injections of a mercurial preparation has resulted in a strongly positive serum being converted into an absolutely negative one as tested with all antigens, including the cholesterinized extracts. The troublesome cases are those showing 25 per cent. or less inhibition of hemolysis with cholesterinized extracts and with complete hemolysis with plain extracts. In such instances, if there is a history of infection, we report the reaction as positive. If there occurs a similar slight inhibition with the plain extracts the case is almost certainly luetic. The most striking results, however, are those showing absolute inhibition of hemolysis with the cholesterinized, and complete hemolysis with the plain extracts. The results would indicate that the majority of these cases are to be regarded as luetic although it is necessary to continue the study into a larger group of cases, controlled by careful histories and autopsies.

Our cholesterinized alcoholic extracts of human and pig heart proved less sensitive than the extract of beef heart; i. e., they yielded but 5 per cent. pseudo-reactions with normal serums (one out of twenty serums tested) and did not yield quite as many positive reactions with the serums tested.

1. In 52.9 per cent. of cases the reactions were equal to those obtained with alcoholic extract of syphilitic liver.

2. In 29.3 per cent. of cases the reactions were stronger.

3. In no case were the reactions weaker.

4. In three cases serums yielded positive reactions with the cholesterinized extracts and were negative with the control extracts.

- One case (+) specific optic neuritis.  
One case (++) tertiary lues.  
One case (+++++) tertiary lues. History positive.

From the fact that we have found these extracts to be less likely to yield pseudo-reactions we rely more on them than the cholesterinized beef heart extract.

(e) Influence of Cholesterin on the Antigenic Properties of Extract of Syphilitic Liver: It may be stated in general that the addition of cholesterin to alcoholic and acetone extracts of syphilitic liver increases the antigenic value so that results with these "antigens" are similar to those obtained with the cholesterinized extracts of heart or normal liver. The addition of cholesterin does not seem to render these extracts any more specific than the cholesterinized extracts of normal organs, and they are just as likely to give minor degrees of inhibition of hemolysis with a small percentage of normal scrums. The addition of cholesterin to a solution of acetone insoluble lipoids in methyl alcohol results essentially in a mixture of lecithin and cholesterin and the sensitivity is increased, but the difference in sensitiveness is not quite so marked as between the plain and cholesterinized alcoholic extracts.

#### SUMMARY

1. The syphilis reaction is probably due to the inactivation of complement by means of a reactionary product, "reagin," or the so-called syphilis antibody, in the presence of a suitable lipid, the phenomenon being known as complement deviation or fixation.

2. While this lipodophilic "reagin" is not entirely a specific product of the *Treponema pallidum*, similar "reagins" being present in the serums of persons infected with yaws and leprosy, it is possible that the question of specificity may be settled by further chemical and biological studies of the lipoids, especially of tissues infected with *Treponema pallidum*. Thus extracts of syphilitic liver possess a higher antigenic value than extracts of normal liver. It is true that "antigens" of normal liver will give a positive reaction in most cases of active syphilis, but the degree of reaction in these same cases and especially with latent or actively treated cases, will be less than with alcoholic extracts of syphilitic liver. The difference is probably due to a difference in the lipid content of the extracts, and it is possible that the *Treponema pallidum* may alter the lipoids of the liver to this extent. Plain alcoholic extracts of human, pig or beef heart possess higher antigenic value than similar extracts of normal liver, due probably to the extraction of more cholesterin. The addition of cholesterin to any or all of these extracts increases their antigenic properties. With acetone-insoluble lipid "antigen" this difference is not so marked, due in part to the fact that such an extract is



composed largely of lecithin, which in itself possesses a high antigenic value.

3. Table 3 expresses the relative values of the various extracts in the practical serum diagnosis of syphilis, taking a suitable alcoholic extract of syphilitic liver as a standard for comparison. It must be observed, however, that the cholesterinized extracts are more sensitive than the plain alcoholic extracts of syphilitic liver.

TABLE 3.—COMPARATIVE ANTIGENIC PROPERTIES OF VARIOUS EXTRACTS

Extracts	Antigenic Properties Compared to Alcoholic Extracts of Syphilitic Liver				
	Equal	Stronger	Weaker	Neg. (pos. with Alc. Ext. Syph. Liver)	Pos. (Neg. with Alc. Ext. Syph. Liver)
Cholesterinized alcoholic extract of human and pig heart .....	52.9	29.3	0.	0.	17.0
Cholesterinized alcoholic extract of beef heart...	45.3	29.4	1.4	0.	23.0
Acetone-insoluble lipoids..	73.0	10.8	0.7	3.2	1.8
Alcoholic extract pig and beef heart .....	71.1	1.9	13.4	5.7	7.6
Acetone extract syphilitic liver .....	68.7	4.4	18.9	6.6	1.3
Alcoholic extract normal liver .....	73.2	0.	23.5	3.5.	0.

In the order of efficiency in the practical serum diagnosis of syphilis we would arrange the various extracts as follows:

1. Cholesterinized alcoholic extracts of human, pig and beef heart, named in the order of efficiency and safety. It should be remembered that these extracts may yield slight degrees of inhibition of hemolysis with normal serums and must therefore be carefully controlled. Cholesterinized alcoholic extracts of syphilitic liver have the advantages of cholesterin and any additional product of the *Treponema pallidum* which may be present.

2. Plain alcoholic extracts of known syphilitic liver.

3. Acetone-insoluble lipoids (Noguchi).

4. Plain alcoholic extracts of human, pig and beef heart.

5. Acetone extract of syphilitic liver.

6. Plain alcoholic extract of normal liver.

Best results are secured in the practical serum diagnosis of syphilis by using several "antigens" with each serum, including plain and cholesterinized extracts. This increases the amount of work and the

quantities of the various components of the reaction, but results warrant the former and the latter is readily overcome by using just half the quantities of Wassermann's original technic. Thus 0.1 c.c. of serum may be used with each "antigen" instead of the usual 0.2 c.c., the quantities of complement, amboceptor and corpuscle suspension being equally divided. In this manner 1.0 c.c. of serum (2 to 3 c.c. blood) will be sufficient for conducting the Wassermann and Noguchi reactions with at least two to four "antigens," including the usual serum controls.

We wish to express our thanks to Dr. Jay F. Schamberg, and also to Dr. W. Hickson of the Vineland Training School, for many serums and careful histories of patients.

Just before sending the manuscript to the publishers we noted the paper of Walker and Swift, *Jour. Exper. Med.*, 1913, xviii, 75, in which they conclude that cholesterinized extracts fulfill the requirements of a standard antigen.

## STUDIES ON THE CIRCULATION IN MAN

### IX. THE BLOOD-FLOW IN THE HANDS (AND FEET) IN CASES IN WHICH OBVIOUS ANATOMICAL DIFFERENCES EXIST \*

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In this paper and succeeding ones I propose to continue the description and discussion of the results obtained in clinical cases already begun<sup>1</sup> in papers VI and VIII.

#### GENERAL REMARKS

A portion of the material studied consisted of patients in the Dispensary of Western Reserve University and Lakeside Hospital, referred to in the protocols simply as the Dispensary. The major portion of the material was from the wards of the City and other hospitals. A few cases were placed at my disposal from the private practice of friends. This is pointed out because comparable results are much more easily obtained in successive observations on hospital patients whose surroundings are fairly constant, than on dispensary or private patients. At any rate, in order to obtain comparable results, fewer precautions are necessary in the case of the hospital patients. In particular, the influence of the external temperature, the factor which of all others among the external conditions is most apt to influence the results, is much more easily controlled in the case of the hospital patients, especially in winter. The observations on the latter were made in a room in the hospital to which the patients were brought in the wheel chair, or the movable bed; or to which, if well enough, they walked. As is true for many kinds of physiological clinical examination, the observations are more satisfactorily made in a separate room than in the open wards. Where the external conditions are controlled and the clinical state of the patient has not altered noticeably, the results of the blood-flow measurements from day to day, or indeed from week to week, show a very fair and often a surprising degree of constancy. Not only is the hospital patient in an environment whose temperature varies comparatively little, but his diet is also controlled and the general régime under which he lives is relatively stable. In many of the hospital cases the approximate constancy of the flow was not only seen in the case of

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\* From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.

\* Submitted for publication July 18, 1913.

1. Stewart, G. N.: Jour. Exper. Med., 1913, xviii, Nos. 2 and 4.

one and the same hand, but also in the proportion between the flows in the two hands where a conspicuous difference existed between the two. As regards the dispensary material, care had to be taken in winter that the person was long enough in the room before the observations were begun, especially if he came to the dispensary with bare hands. It is true that if the observations were immediately begun a result would be obtained which would be correct enough for the given condition of the hands, but it would be useless as an expression of the flow under the approximately standard conditions which have to be established if comparison of the flow in the same patient at different times or in different patients is proposed. In summer the factor of external temperature presents no greater difficulty in dealing with dispensary than with hospital patients.

In addition to the clinical interest which attaches to the measurements in the individual cases dealt with in this paper, the material was chosen partly as a test of the technic of the method, as in many of the cases the qualitative difference between the two hands could be foreseen from the anatomical difference which existed.

In Steve S., a man 21 years old, with healing burns on both hands, but more extensive on the right, the flow was 7.04 grams per 100 c.c. per minute for the right hand and 5.44 grams for the left hand (for the last nine minutes in the calorimeters), with an average room temperature of 25. The burned areas on both hands were quite red, and taking the whole surface of the hands into account the vascularity of the superficial layers must have been considerably greater in the right than in the left hand. In both hands many blood-vessels in the new tissue must be nearer the surface than in the normal hand, a point of interest in connection with the technic of the method.<sup>2</sup> In spite of the red color, both hands feel rather cold to the touch, and this agrees perfectly with the tardiness<sup>3</sup> with which the thermometers rose at first and with the calculated blood-flow, which for the man's age and the room temperature, is subnormal. The redness is due to the number of capillaries in the healing areas and the fact that they lie just under the surface, not to a great flow of blood through widely dilated vessels. The case presented the opportunity of

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2. Heart, 1911, iii, 55.

3. This tardiness is expressed in the calculated flow for the first eight minutes of immersion in the calorimeters, which is only 3.74 grams per 100 c.c. per minute for the right, and 2.49 grams for the left hand. The fact that the ratio of the flow in the left hand to that in the right is 1:1.50 for the first eight minutes, while it is 1:1.29 for the last nine minutes, in the calorimeters, indicates clearly that the increase in flow in the last period was not due to a central cause (increase in the driving power of the heart) which would have affected the two hands equally, but to a peripheral cause (removal of vasoconstriction which happened to be proportionately greater in the left hand at the beginning of the immersion in the calorimeters than later on).



studying the flow in blood-vessels which have not long existed and whose vasomotor connections may be still incomplete or immature. Immersion of the left hand in warm water caused a good increase, and subsequent immersion of the left hand in cold water a marked decrease in the flow in the right hand. The persistence of both reflexes was remarkable. Another peculiarity not observed in any other case was that the temperature of the right hand calorimeter during the immersion of the left hand in the warm or cold water, showed abrupt changes, for instance,

TABLE 1.—CALORIMETER TEST OF STEVE S.

Time	Right	Left	Room Temp. C.	Time	Right	Notes
3:33½	29.92	29.84	.....	3:55	30.32	
3:37	29.90	29.88	.....	3:56	30.37	Room 24.8 C.
3:38	29.91	29.90	25.3	3:57	30.495	
3:39	29.92	29.905	.....	3:58	30.505	
3:40	29.93	29.915	.....	3:59	30.53	
3:41	29.95	29.925	.....	4:00	30.64	
3:42	29.97	29.925	.....	4:01	30.64	
3:43	29.99	29.93	25.0	4:02	30.67	
3:44	30.01	29.935	.....	4:03	30.75	
3:45	30.015	29.945	.....	4:04	30.82	Room 24.9 C.
3:46	30.05	29.97	.....	4:05	30.86	
3:47	30.10	29.99	.....	4:06	30.88	At 4:06 left hand put in water at 10 C.
3:48	30.11	30.025	.....	4:07	30.87	
3:49	30.16	30.05	.....	4:08	30.88	Room 24.8 C.
3:50	30.20	30.07	24.6	4:09	30.89	
3:51	30.215	30.12	.....	4:10	30.99	
3:52	30.28	30.14	.....	4:11	30.98	
3:53	30.295	30.15	.....	4:12	30.97	
3:54	30.305	30.18	.....	4:13	30.98	
.....	.....	.....	.....	4:14	30.995	Hand out of cal. at 4:14.
.....	.....	.....	.....	4:27	30.88	At 4:27 L. is at 29.94 C. Room 24.2 C.

Cooling of calorimeters, R., 0.115 degrees in thirteen minutes; L., 0.24 degrees in thirty-three minutes. Volume of right hand in calorimeter, 328 c.c.; left, 340 c.c. The hands, especially the right, could not be made to hang vertically in the calorimeters owing to adhesions. The volumes given represent the portions of the hands actually inside the calorimeters. Water equivalent of calorimeters with contents, R., 3,357; L., 3,367. Rectal temperature, 37.3 C.

a sudden rise of the thermometer between the third and fourth minutes of immersion of the left hand in the cold water — not succeeded by any further rise. The patient denied that his hand ever touched the thermometer, and from the position of the thermometer in the calorimeter contact of the hand with it can scarcely occur, unless the hand is executing extensive groping movements, of which there was no sign in these observations. The most plausible explanation of the phenomenon would

seem to be that in the new blood-vessels, although the vasomotor connections had been established, there is still an abnormal vasomotor instability. The intensity of the total reaction may be due either to hyperexcitability of the receptive surfaces of the left hand (afferent path) or to hyperexcitability of the newly-formed vasomotor endings including the muscle of the new blood-vessels (efferent path) or to both. The warm water was purposely made 3 degrees lower, and the cold water 2 degrees higher than usual, lest pain should be caused. But he did not feel any pain.

Steve S., aged 21, a sailor, was burned November 4, 1911, by the bursting of a steam pipe on a steamer. He was unconscious for a fortnight. The face, including the ears, the neck, the back, the knees and both hands and arms were burned. The sight of the right eye was destroyed. He was admitted to the City Hospital Feb. 19, 1912. Both forearms and a small portion of the upper arms above the elbow were denuded of skin and covered with jelly-like exudation and granulations. Physical examination of the heart and lungs was negative.

The blood-flow in the hands was measured April 5, 1912, at which time healing was pretty well completed, although there was still some suppuration on the right forearm, a little above the wrist. The burn on the right hand involved the palms as well as the back of the hand and there are considerable adhesions between the fingers, rendering the hand practically useless. The left palm escaped in great measure, but the back of the hand was burned. The burned areas on the hands were still quite red. Hands in bath at 3:24 p. m., in calorimeters at 3:34½; 3,015 c.c. of water in each calorimeter.<sup>4</sup> At 3:54 p. m. left hand put into water at 40 C. Pulse 80.

In Frank P., a man 42 years old, suffering from a malignant tumor of the right forearm, with some edema of the forearm down to the wrist, the flow in the right hand was 4.69 grams per 100 c.c. of hand per minute, in the left hand 5.31 grams, with an average room temperature of 23.2 C. The deficiency in the right hand was an actual deficiency and not merely an apparent one (owing to the reckoning in of edema liquid in the volume of the hand), since by measurement the right hand was only 3 c.c. larger than the left. It is therefore probable that the deficiency is due to the interference of the tumor with the venous return from the hand. Insofar as it is not connected with inflammatory changes, the edema is also probably caused by interference with the venous flow. In certain cases the blood-flow measurement might help to settle the question whether edema is due to obstruction on the lymph path or on the venous path. Obstruction confined to the lymphatics might cause edema without diminution in the blood-flow. Where a tumor is confined to the bone, obstruction to the flow of blood in the hand may be expected to be less marked than where it has extensively invaded the soft tissues.

Frank P., a carpenter, aged 42, was admitted to the City Hospital July 18, 1912, with a tumor mass about 10 cm. in diameter on the outer surface of the right forearm. The mass was slightly fluctuant, tender on pressure and attached

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4. In all observations on the hands unless otherwise stated, this was the quantity of water in each calorimeter.

to the bone. There was some edema in the right forearm and wrist. There was another tumor mass over the upper third of the left tibia about 15 cm. in diameter. It had been incised for drainage and a muco-purulent fluid was being discharged. There was a small mass and tenderness over the upper third of the right fibula. X-ray examination showed a large rounded mass on the right forearm going deep into the tissues, as much of it below the surface as above the surface. The swellings on the left leg and upper forearm were noticed about the same time, three months before admission. They have gradually increased in size and of late have been very painful. Physical examination of the heart was negative. Thorax emphysematous and expiration prolonged. Percussion note raised in pitch and shortened on the left side, especially at the left base. Respiratory sounds diminished on the left side, where numerous rhonchi were present. Tactile fremitus diminished on the left side. The diagnosis was multiple sarcoma. The patient refused operation and left the hospital. The examination of the blood-flow was made Aug. 1, 1912. Hands in bath at 2:22 p. m., in calorimeters at 2:33 and out of calorimeters at 2:52. Pulse (sitting) 96. The day was exceptionally cool for the season.

TABLE 2.—CALORIMETER FINDINGS IN CASE OF FRANK P.

Time	Right	Left	Room Temp. C.	Time	Right	Left	Room Temp., C.
2:32	30.16	30.14	....	2:44	30.48	30.49	23.4
2:34	30.18	30.15	22.9	2:45	30.505	30.54	....
2:35	30.205	30.175	....	2:46	30.53	30.58	23.2
2:36	30.23	30.20	23.1	2:47	30.57	30.62	....
2:37	30.26	30.21	....	2:48	30.595	30.665	....
2:38	30.27	30.26	23.1	2:49	30.61	30.69	....
2:39	30.315	30.29	....	2:50	30.64	30.72	23.0
2:40	30.35	30.33	23.1	2:51	30.67	30.75	....
2:41	30.39	30.37	....	2:52	30.695	30.765	22.95
2:42	30.41	30.43	....	3:09	30.485	30.565	....
2:43	30.44	30.46	....	....	.....	.....	....

Cooling of calorimeters in seventeen minutes, R., 0.21 degrees; L., 0.20 degrees. Volume of right hand, 476 c.c.; the same for a second measurement. Volume of left hand, 473 c.c. To the eye the right hand appeared somewhat swollen, but this seemed to be an error of judgment, caused by the distinct swelling of the right forearm below the level of the tumor down to and involving the right wrist. The swelling did not descend below the wrist. Water equivalent of calorimeters with contents, R., 3,476; L., 3,473. Blood-pressure, left arm, systolic 115 (palpation), 114 (stethoscope), 80 (sound gone). Pulse (sitting) 114. Rectal temperature, 37.95 C.

A condition in which edema coincided with obstruction to the lymph-flow not involving the venous flow seems to have been realized in the case of Charles B., a man 47 years old, in whom the diagnosis of Hodgkin's disease was made. He entered the hospital with both legs and feet greatly swollen. The swelling was stationary and little if at all diminished in the morning. During his entire stay in the hospital (over five weeks) the swelling of the legs remained unchanged. There was no edema elsewhere. All accessible lymph-nodes were enlarged. The blood-flow in the right foot was 2.34 grams per 100 c.c. of foot per minute, or, allowing for

the edema fluid, 3.04 grams, and in the left foot, 2.50 grams (allowing for the edema fluid, 3.24 grams) with average room temperature 22.5 C. These flows are by no means small for the feet. Indeed, in comparison with the hand flows (2.82 grams for the right and 3.22 grams for the left hand) they are large. A certain degree of anemia, some cyanosis and dyspnea were present, and this may contribute to the small hand flow.<sup>5</sup> It may be supposed that this would tell equally on the blood-flow through the feet, but in the absence of special observations on this point, we cannot be certain whether the compensatory vasoconstriction in anemia is not greater in the anterior limb than in the posterior limb. It is conceivable that the circulation in the anterior limb has a closer association with the pulmonary circuit than the circulation in the posterior limb. That the hands were exceptionally liable to vasoconstriction was shown by observations on Nov. 27, 1912, which gave a still smaller flow. It is rare to find a case in which the flow per 100 c.c. of foot per minute comes out as great as the flow per 100 c.c. of hand per minute in the same individual. In the vast majority of clinical cases the hand flow greatly preponderates, and this is the invariable rule for normal persons so far as our experience goes.<sup>6</sup>

Interference with the local circulation would seem to be excluded in this case as a cause of the edema of the legs. It is more likely, indeed, if we take account of the hand flows, that there is a local acceleration of the blood flow in the legs, perhaps through partial paralysis of their vasoconstrictors by the pressure of the same masses, if it is an affair of mechanical pressure, as are responsible for the edema. If we confine ourself to the question, leaving out all other possibilities, whether the edema is dependent on venous or on lymphatic obstruction, the blood flow measurements give an answer in favor of lymphatic obstruction, to this extent supporting the diagnosis of Hodgkin's disease.

Charles B., lumberman, 47 years old, height 5 feet, 6 inches, was admitted to City Hospital November 8, 1912. The glands were generally enlarged, one of the inguinal glands on the left side being as big as a walnut. Both legs were edematous and covered with varices. The varicose veins, however, were of a good many years' standing, and had not increased during the present illness, which began two months before admission with pain in the back and shortness of breath. About two weeks before admission the pain recurred and he almost fainted with the pain in the back. The feet began to swell two days before admission; the left leg began to swell two days later than the right. No edema elsewhere than in the legs. He had always lived in Cleveland or the neighborhood. Has never had piles. The pain in the back was a cutting pain on both sides. There had never been gravel in the urine. The skin and mucous membranes showed slight anemia, slight cyanosis, no icterus. Thorax barrel-shaped, moves slightly more on the right side. The costal angle is obtuse, the breathing mainly abdominal. Further examination of thorax and the heart, also of

5. Stewart: Paper VI, Jour. Exper. Med., 1913, xviii, No. 2.

6. Stewart, G. N.: Paper VII, Jour. Exper. Med., 1913, xviii, No. 4.



the abdomen and rectum, negative. X-ray examination of abdomen negative. Some sclerosis of the radial artery. The reflexes were active. The urine contained albumin with a few granular casts on November 9, a trace only of albumin on November 18. On November 14 the leukocyte count was 14,000 (polymorphonuclear neutrophils 70 per cent., eosinophils 4.5 per cent., large mononuclears 5.5 per cent., small mononuclears 15 per cent., transitionals 2.5 per cent.).

TABLE 3.—CALORIMETER FINDINGS IN CHARLES B. (FEET)

Time	Right	Left	Room Temp., C.	Time	Right	Left	Room Temp., C.
2:30	31.24	31.28	.....	2:47	31.38	31.465	.....
2:33	31.16	31.23	.....	2:49	31.40	31.485	22.7
2:35	31.19	31.27	.....	2:51	31.43	31.53	22.9
2:37	31.22	31.30	21.5	2:53	31.465	31.565	22.5
2:39	31.25	31.325	21.9	2:55	31.49	31.58	.....
2:41	31.29	31.365	22.1	2:57	31.42	31.55	.....
2:43	31.32	31.395	22.2	3:14	31.04	31.16	.....
2:45	31.335	31.44	22.4	....	.....	.....	.....

Cooling of calorimeters in seventeen minutes, R., 0.38 degrees; L., 0.39 degrees. Volume of right foot 1,313 c.c., of left foot 1,313 c.c. His feet were naturally not large, requiring a No. 7 shoe. Water equivalent of each calorimeter with contents, 3.694.

Hands in bath at 3:17 p. m. in calorimeters at 3:26½ and out of calorimeters at 3:45.

TABLE 4.—CALORIMETER FINDINGS IN CHARLES B. (HANDS)

Time	Right	Left	Room Temp., C.	Time	Right	Left	Room Temp., C.
3:25	30.80	30.73	.....	3:37	30.805	30.765	23.5
3:28	30.77	30.705	21.8	3:38	30.805	30.77	22.9
3:29	30.775	30.71	21.9	3:39	30.81	30.78	22.8
3:30	30.775	30.73	.....	3:40	30.815	30.79	22.7
3:31	30.78	30.735	22.3	3:41	30.825	30.82	22.6
3:32	30.78	30.735	22.5	3:42	30.83	30.83	22.7
3:33	30.79	30.745	23.0	3:43	30.84	30.84	.....
3:34	30.795	30.745	23.4	3:44	30.85	30.85	.....
3:35	30.795	30.755	23.7	3:45	30.86	30.855	.....
3:36	30.80	30.755	23.7	3:53	30.75	30.75	.....

Cooling of calorimeters in eight minutes; R., 0.11 degrees; L., 0.105 degrees. Volume of right hand 480 c.c.; of left 462 c.c. Water equivalent of calorimeters with contents, R., 3.479; L., 3.465. Rectal temperature 36.9 C. Blood-pressure in left arm (Nov. 27, 1912), systolic 158 (palpation), 155 (stethoscope), 95 (sound gone).

The blood-flow in the feet and hands was measured Nov. 29, 1912. The swelling of the legs, the patient said, was stationary. The varicose veins could not be seen on account of the swelling. There was no pain in the back, but he had pain in the lower abdomen a little above the level of the groin when he walked any distance. Pulse (sitting) 98. Feet in bath at 2:06½ p. m.; in

calorimeters at 2:31 and out of calorimeters at 2:55½: 2.575 c.c. of water in each calorimeter, as finally reduced in size.<sup>7</sup>

TABLE 5.—CALORIMETER MEASUREMENTS OF FEET IN CHARLES W.

Time	Right	Left	Room Temp., C.	Time	Right	Left	Room Temp., C.
2:16	31.30	31.38	.....	2:43	31.35	30.92	.....
2:19	31.24	31.26	23.8	2:45	31.38	30.93	23.6
2:21	31.225	31.265	.....	2:47	31.41	30.945	23.4
2:23	31.21	31.11*	.....	2:49	31.44	30.955	23.8
2:25	31.21	31.055	23.3	2:51	31.475	30.975	24.1
2:27	31.205	31.02	.....	2:53	31.505	30.995	24.1
2:29	31.21	30.98	23.15	2:55	31.535	31.025	23.6
2:32	31.22	30.94	23.3	2:57	31.575	31.045	23.2
2:35	31.24	30.93	.....	2:59	31.60	31.07	.....
2:37	31.275	30.92	23.5	3:01	31.57	31.05	.....
2:39	31.295	30.92	24.0	3:14	31.37	30.87	.....
2:41	31.32	30.92	23.9	....	.....	.....	.....

\* Stirring in L. had not been thorough till now for fear of hurting the foot.

Cooling of calorimeters in thirteen minutes: R., 0.20 degrees; L., 0.18 degrees. Volume of right foot 1.203 c.c., of left foot 1.103 c.c. Water equivalent of foot calorimeters with contents, R., 3,817, L., 3,747. Hands in bath at 3:24½ p. m., in calorimeters at 3:34 and out of calorimeters at 3:45.

TABLE 6.—CALORIMETER MEASUREMENTS OF HANDS OF CHARLES W.

Time	Right	Left	Room Temp., C.	Time	Right	Left	Room Temp., C.
3:33	31.27	31.22	.....	3:41	31.67	31.605	.....
3:35	31.29	31.255	22.9	3:42	31.72	31.66	24.2
3:36	31.33	31.31	22.9	3:43	31.785	31.72	24.1
3:37	31.40	31.36	23.0	3:44	31.83	31.765	.....
3:38	31.465	31.425	23.25	3:45	31.895	31.82	.....
3:39	31.53	31.485	23.5	3:52	31.79	31.72	.....
3:40	31.60	31.55	23.9	....	.....	.....	.....

Cooling of hand calorimeters in seven minutes: R., 0.105 degrees; L., 0.10 degrees. Volume of right hand 486 c.c., of left hand 470 c.c. He is right handed. There is no difference, he says, in his hands, as to strength or otherwise, nor was there any difference at the time of the "strokes." Water equivalent of hand calorimeters with contents, R., 3,484, L., 3,471. Rectal temperature 37.9 C.

He was discharged Dec. 22, 1912, able to use the left foot better, he says, than for the previous seven years.

Charles W., a clerk, aged 42, was admitted to the City Hospital October 29, 1912. He had rheumatism at the age of eight years, since which time the left leg seemed to be weaker and smaller than the right. At that time the arms and legs were somewhat swollen and extremely sensitive. He had a stroke of paralysis in 1903. He was sitting in a chair reading and found he could not

7. For details of the method of measuring the flow in the feet see Paper VII of this series.<sup>8</sup>

rise to walk. There were no convulsions. Both arms and legs were affected. He could not move the eyes, nor talk, and his sight was affected. No bowel or bladder trouble. In seven months he had recovered from the stroke and had no trouble in walking or in any other way until 1907, when he had a second stroke of the same kind preceded by slight dizziness, but otherwise no subjective signs. There was no disturbance of vision. It took four years for his complete recovery from the second attack and then he felt "real well." Four months before admission he fell on the sidewalk and abraded the left shin about 3 inches. It healed somewhat and the scab "raised up" and began to pain him severely, the pain shooting down the leg. He went to a hospital for four weeks and it again healed to some extent. On leaving the hospital it became worse, then healed again, but continued to be very painful. It opened again and the pain continued so that he could not sleep. There are many petechial spots on the left leg. Examination of thorax negative. Reflexes increased in the left leg. The left leg is atrophied and much smaller than the right and  $1\frac{1}{2}$  inches shorter. The left foot is not small, although doubtless smaller than the right. It is the lower leg which is conspicuously small. He denies lues emphatically. The Wassermann blood test was twice negative. Spinal fluid also negative, cell count negative. Noguchi negative.

The blood-flow in the feet and hands was measured Dec. 18, 1912. There was no pain in left leg. Feet in bath at 2:04 p. m., in calorimeters at 2:17½ and out of calorimeters at 2:59; 2,775 c.c. of water in each calorimeter. Pulse (sitting) 92. He felt the feathers equally well on both legs.

I was asked to make an examination of the flow in the feet in case some light might thus be thrown on the diagnosis. The flow in the right foot was 1.89 grams per 100 c.c. per minute and in the left 1.56 grams (for the last ten minutes in the calorimeters), with an average room temperature 23.9 C. From the small deficiency in the flow in the left foot as compared with the right, it was concluded that there was no obstruction in the main arterial supply of the left leg which could account for the slow healing of the sore and for the pain in the leg. If the cause was vascular it was therefore a vasomotor affair, possibly even a local vasoconstriction due to the pain itself. Undue susceptibility to vasoconstriction might account for the relatively small flow in both feet. The slow increase in the flow in the left foot at the beginning of the observations supports the view that it was peculiarly susceptible to vasoconstriction. The consequence of this stubborn vasoconstriction is that the ratio of the flow in the left foot to that in the right for a period of twenty-four minutes in the calorimeters, beginning seventeen and one-half minutes from the first insertion therein, is 1:1.55, while for the last ten minutes in the calorimeters it is 1:1.21. The cause of the small flow was not a central one (deficiency in the driving power of the heart), since the flow in the hands was normal (10.62 grams for the right hand per 100 c.c. per minute and 10 grams for the left with room temperature 23.6 C.).

Philip D., a street laborer, 47 year old, a dispensary patient. Weight 170 pounds; height 5 feet, 10½ inches. His right arm was shorter than the left from inflammatory rheumatism, he said, 13 years previously. Some arteriosclerosis. He had had pneumonia, pleurisy and rheumatism. He had a troublesome cough and coughed a good deal while his hands were in the calorimeters.

He had a great many clothes on but the hands were not warm. The blood-flow in the hands was examined Dec. 20, 1910. At 2:23 p. m. hands put in bath; at 2:32 in calorimeters, and at 2:45 taken out of calorimeters. He was in a hurry to get back to work and therefore his hands were left only nine minutes in the bath; 3.050 c.c. of water in each calorimeter. This was one of the earlier cases examined and the calorimeter and room temperature was 19.1 C. Bath temperatures were lower than are usually employed now.

TABLE 7.—CALORIMETER MEASUREMENTS IN CASE OF PHILIP D.

Time	Right	Left	Time	Right	Left
2:30	29.49	29.44	2:40	29.50	29.50
2:33	29.40	29.38	2:41	29.53	29.61
2:34	29.41	29.38	2:42	29.59	29.60
2:35	29.40	29.38	2:43	29.61	29.63
2:36	29.41	29.40	2:44	29.66	29.70
2:37	29.41	29.40	2:45	29.69	29.70
2:38	29.43	29.42	2:55	29.54	29.56
2:39	29.47	29.45	....	.....	.....

Cooling of calorimeters in 10 minutes: R., 0.15 degrees; L., 0.14 degrees. Volume of right hand in calorimeter, 385 c.c., of left, 375 c.c. Pulse (standing) 68. Mouth temperature 37.0 C.

The flow in the right hand, which showed no wasting, was 7.05 grams per 100 c.c. per minute; in the left 7.41 grams, with the rather low room temperature of 19.1 C. These flows are fair for his age and clinical condition. As his haste to return to work did not permit a sufficiently long examination, and no opportunity to repeat the observations occurred, it is not possible to say whether the small deficiency in the right hand flow is permanent and associated with the shortening of the arm.

Some interesting cases in which obvious differences between the two hands exist were found in unilateral inflammations. Of these we shall cite three—one in which the inflammation was due to an infected finger, one in which it was due to gout, and a third in which it was due to a sprain.

Charles C., a teamster, aged 36, had an infection of the right hand, with considerable swelling and redness; otherwise he was in good health. The protocol has been already published.<sup>8</sup> The flow was 11.93 grams per 100 c.c. per minute, or allowing for the edema fluid, 13.05 grams in the right hand against 4.92 grams in the left, with room temperature at 23.8 C.

There can be no doubt that along with an increase in the flow in the inflamed hand in this case there goes a decrease in the flow in the normal hand, since 4.92 grams is an abnormally small flow for a healthy man of this age. The suggestion is that in order to provide for the increased flow in the infected area a vasoconstriction, possibly elicited reflexly through the pain nerves of the infected hand, is brought about

8. Heart, 1911, iii, 82.



elsewhere and particularly in the symmetrically placed parts on the opposite side.

However this may be, a fact of interest emerges when the vasomotor reflexes from the left to the right hand are studied. Immersion of the left hand in cold water caused scarcely any reduction of the flow in the right hand (from 11.93 to 11.15 grams, or allowing for the edema liquid, from 13.05 grams to 12.20 grams). This is a very much smaller reaction than normal, and suggests that vasoconstrictor impulses to the inflamed part are blocked, perhaps, by a local action of the bacterial products or of something produced in the reaction between them and the leukocytes on the vasomotor nerve endings. It is clear that such a block may be of importance in maintaining against casual and vagrant vasoconstrictor impulses, so to say, the full stream of blood which is so important a factor in combating the infection.

Immersion of the left hand in warm water also caused hardly any increase in the flow through the right hand beyond promptly removing the small effect of the cold water. But this is of less significance, as the vasodilatation in the infected hand was probably already nearly maximum, and it is usual to obtain a relatively small reflex vasodilatation in a hand with a large initial flow. It is, however, again of interest that the initial vasoconstriction which normally follows immersion of the contralateral hand in warm water is here absent, a further indication of a block on the vasoconstrictor path. A different picture is presented by the case of gouty (non-bacterial) inflammation in which two examinations were obtained.

Harry S., aged 50, a patient at the City Hospital. He had suffered from gout for twenty years. He had no knowledge of its having been in the family before him. His mother suffered much from rheumatism. There are tophi in the scrotum as well as in the ears. There is a trace of albumin in the urine, also some fine granular and hyaline casts. First examination of the hand flow April 3, 1912. The right wrist and hand were painful and swollen. Hands in bath at 2:20 p. m., in calorimeters at 2:32 p. m. Mouth temperature 37.1 C. At 2:44 p. m. left hand put in water at 43 C.

Second examination of hand flow July 9, 1912. The right wrist and hand were much swollen and there was considerable pain. The day was warm and muggy. Pulse (sitting) 100. Hands in bath at 2:07 p. m.; in calorimeters at 2:17 p. m. Only 2,965 c.c. of water was put into each calorimeter on account of the swelling of the right hand. At 2:30 p. m. left hand put into water at 8.3 C. He feels it very cold.

At the first examination with room temperature 24.3 C., the flow was 6.88 grams per 100 c.c. of hand per minute (or allowing for the swelling, 7.47 grams) in the right hand and 3.07 grams in the left. Immersion of the left hand in warm water caused the usual initial diminution of the flow in the right to 4.66 grams (or, allowing for swelling, 5.06 grams), followed by an increase to 9.88 grams (or, allowing for the edema, 10.73 grams), a good vasodilatation. It must, of course, be remembered that the initial flow in the right hand did not correspond with anything like

a maximal vasodilatation. It is therefore all the more significant that immersion of the left hand in cold water caused a marked and persistent decline of the flow in the right hand to 3.84 grams (or, allowing for the swelling, to 4.16 grams). There is, then, in this case of non-bacterial inflammation, no sign of vasoconstrictor block.

TABLE S.—CALORIMETER MEASUREMENTS IN CASE OF HARRY S.

Time	Right	Left	Room Temp., C.	Time	Right	Notes
2:33	30.32	30.21	.....	2:51	31.11	At 2:51 left hand put in water at 8 C.
2:34	30.36	30.22	.....	2:52	31.13	
2:35	30.37	30.23	.....	2:53	31.18	
2:36	30.40	30.23	24.2	2:54	31.19	Room at 24.0 C.
2:37	30.43	30.23	.....	2:55	31.21	
2:38	30.49	30.24	.....	2:56	31.23	
2:39	30.55	30.27	.....	2:57	31.245	
2:40	30.59	30.305	24.3	2:58	31.27	At 2:59 left hand dried and wrapped. Room at 24.1 C.
2:41	30.63	30.315	.....	2:59	31.29	
2:42	30.69	30.33	.....	3:00	31.295	
2:43	30.71	30.33	.....	3:01	31.32	At 3:05 hand out of calorim. Room 32.7 At 3:13½ L. is at 30.07 C.
2:44	30.76	30.34	.....	3:02	31.33	
2:45	30.795	.....	.....	3:03	31.37	
2:46	30.805	.....	24.5	3:04	31.395	
2:47	30.86	.....	.....	3:05	31.42	
2:48	30.92	.....	.....	3:13½	31.33	
2:49	30.99	.....	.....	.....	.....	
2:50	31.06	.....	24.2	.....	.....	

Cooling of calorimeters: R., 0.09 degree in 81½ minutes; L., 0.27 degree in 29½ minutes. Volume of right hand in calorimeter 456 c.c., of left hand 404 c.c. Pulse (sitting) 76. Water equivalent of calorimeters with contents. R., 3,460, L., 3,418.

The same fact comes out fully as well in the second examination, which was made on a very warm day with a room temperature of 29.7 C. The right hand was much more swollen at the second examination than at the first. At neither examination was there any pain in the left hand. The initial flow was 13.27 grams per 100 c.c. per minute (taking only the flow corrected for the edema liquid) in the right and 8.13 grams in the left hand. As compared with the previous examination, the flow in the left hand is, of course, proportionately more increased by the high external temperature than that in the right. It is still, however, for the temperature a distinctly subnormal flow. On immersion of the left hand in cold water, the flow in the right was diminished to 8.47 grams, a very fair reaction in the normal direction, and this was augmented to 12.82 grams, nearly the initial flow, on subsequent immersion of the left hand in warm water.

TABLE 9.—SECOND EXAMINATION IN CASE OF HARRY S.

Time	Right	Left	Room Temp., C.	Time	Right	Notes
2:16	31.39	31.30	29.7	2:34	32.535	The cold water does not feel as bad as it did.
2:18	31.47	31.42	.....	2:35	32.58	
2:19	31.55	31.445	.....	2:36	32.61	
2:20	31.625	31.48	.....	2:37	32.66	He says the cold water is now more tolerable.
2:21	31.70	31.525	29.65	2:38	32.69	
2:22	31.78	31.56	.....	2:39	32.74	At 2:39 left hand put in water at 42.8 C. Room 29.6 C.
2:23	31.87	31.625	.....	2:40	32.795	
2:24	31.94	31.66	.....	2:41	32.825	Room 29.9 C.
2:25	32.03	31.73	29.8	2:42	32.89	
2:26	32.105	31.77	.....	2:43	32.955	
2:27	32.18	31.82	.....	2:44	33.01	Room 30.0 C.
2:28	32.24	31.855	.....	2:45	33.07	
2:29	32.32	31.90	.....	2:46	33.125	
2:30	32.38	31.95	.....	2:47	33.195	At 2:48 hand out of calorimeter.
2:31	32.41	.....	.....	2:48	33.25	
2:32	32.47	.....	.....	3:02	33.16	At 3:02 temperature of L. is 31.825 C.
2:33	32.50	.....	29.7	....	.....	

Cooling of calorimeters: R., 0.09 degrees in fourteen minutes; L., 0.125 degrees in thirty-two minutes. Volume of right hand in calorimeters 530 c.c., of left hand 410 c.c. Rectal temperature 37.95. Water equivalent of calorimeters with contents: R., 3.519; L., 3.423. Blood-pressure left arm, systolic 114.5 (palpation), 114 (stethoscope), 85.5 (sound gone). Another observation with stethoscope, systolic 108, 88 (sound gone).

It would not be profitable to speculate on the reasons for the difference in the reflex vasomotor reactions in the two cases related further than to point out that the local infection is an acute affair, a bacterial invasion fraught with imminent peril to the organism if it be not promptly dealt with. The mechanism exists also, and in the blood, for the effective and rapid destruction of the bacteria. On the other hand, if the essential condition of the gouty paroxysm be the deposition of urates in the tissue, while an increase in the blood-stream, which does take place, may doubtless be of value in aiding the tissue to segregate the deposit so as to minimize its action as a mechanical irritant, no increase in the blood flow, however great and however sustained, will soon effect the re-resolution of the deposit, which in any case is quite indifferent to the body as a whole and exposes it to no danger of invasion.

The third case was that of Martin B., a man aged 66 years, who had sprained the little finger of his left hand three days before the examina-

tion. The hand was swollen, but not greatly. He said the pain had diminished. The difference in flow between the two hands is very striking (1.7 grams per 100 c.c. per minute for the right hand and 5.2 grams for the left with a room temperature of 23.4 C.). Absolutely the flow is small in both hands, which is accounted for by the arteriosclerosis,<sup>9</sup> the renal disease and the age of the patient. It may be worth pointing out once more that the high systolic arterial pressure far from indicating a copious flow, is commonly the index of a high peripheral resistance, coupled with a small flow. No observations were made on the vasomotor reflexes, although it would have been interesting to compare a case of traumatic non-bacterial inflammation with the case of infection (Charles C.). The complicating conditions, however, especially the arteriosclerosis, which tends to prevent marked vasomotor reflexes, rendered the case an unfavorable one for these tests. Yet there is little doubt that the small flow in the right hand is in part due to reflex vasomotor stimulation, probably from the other hand (pain stimulation?).

Martin B., a carpenter, aged 66, height 5 feet, 8 inches, weight 165 pounds, rather stout, born in Bavaria, but long in this country. Drinks considerable quantities of beer. He has been coming to the dispensary for some time. Extract from dispensary record Nov. 25, 1910: "Marked arteriosclerosis. Albumin in urine, also hyaline casts, epithelial cells, mucus and blood. No sugar. Puffiness under the eyes. Boundaries of heart dulness, upper border of third rib, right sternal margin, and 2½ fingers outside the left nipple. Emphysema." On Jan. 16, 1912, he fell and hurt his left hand, spraining the little finger. The left hand is swollen, but not greatly. He says the pain is better than it was. The blood-flow in the hands was measured Jan. 19, 1911. Systolic blood-pressure 151. Hands put into bath at 2:58 p. m., into calorimeters at 3:10 p. m., and out of calorimeters at 3:24; 3,050 c.c. of water in each calorimeter. Mouth temperature 37.38 C.; room 23.2 C.

TABLE 10.—CALORIMETER MEASUREMENTS IN CASE OF MARTIN B.

Time	Right	Left	Time	Right	Left
3:09	29.49	29.33	3:19	29.49	29.60
3:12	29.45	29.33	3:20	29.50	29.66
3:13	29.45	29.38	3:21	29.50	29.69
3:14	29.46	29.41	3:22	29.52	29.74
3:15	29.46	29.45	3:23	29.53	29.79
3:16	29.47	29.49	3:24	29.54	29.84
3:17	29.48	29.52	3:42	29.38	29.67
3:18	29.48	29.57	....	.....	.....

Cooling of calorimeters in 18 minutes: R., 0.16 degrees; L., 0.17 degrees. Volume of right hand 490 c.c., of left hand 497 c.c. He is right handed. Water equivalent of calorimeters with contents, R., 3,522, L., 3,528. Room temp., 23.6 C.

The influence of pain in diminishing the flow in the hands when the inflamed area is not situated in the hands themselves, is perhaps illustrated in the case of George S.

9. See Paper XI (unpublished) of this series, *THE ARCHIVES INT. MED.*, 1914.



George S., a teamster, aged 37 years, was admitted at the dispensary Jan. 31, 1911, suffering from an inflamed right elbow (acute articular rheumatism). He first felt pain in the elbow three weeks previous to admission suddenly while he was sitting in a saloon. The swelling came on afterwards. He had no knowledge of any injury. The elbow on admission was hot and swollen. He had used alcohol and tobacco to excess. He had delirium tremens in July, 1910. He had chancre four years previously, but denied gonorrhea. He had swelling of four toes of left foot for three days four months previously. The heart was normal. On February 2 the arm was not quite so red, and on February 6 it was much better. The hand and forearm were quite unaffected. His height was 5 feet, 7 inches; weight 156 pounds.

The flow in the hands was examined Feb. 2, 1911. The day was cold and he had no gloves on when he came to the dispensary. Pulse (sitting) 92. Hands in bath at 2:40 p. m., in calorimeters at 2:50 and out of calorimeters at 3:06. The experiment was shorter than had been intended as he said he was suffering so much pain that he could not keep the right hand in any longer. There was 3,050 c.c. of water in each calorimeter.

TABLE 11.—CALORIMETER EXAMINATION IN CASE OF GEORGE S.

Time	Right	Left	Room Temp., C.	Time	Right	Left	Room Temp., C.
2:49	29.74	29.79	.....	3:00	29.75	29.76	22.3
2:51	29.69	29.73	.....	3:01	29.77	29.80	.....
2:53	29.69	29.73	22.2	3:02	29.79	29.80	.....
2:54	29.70	29.74	.....	3:03	29.80	29.81	.....
2:55	29.69	29.73	.....	3:04	29.82	29.83	22.3
2:56	29.70	29.74	.....	3:05	29.83	29.83	.....
2:57	29.71	29.74	.....	3:06	29.85	29.86	.....
2:58	29.72	29.74	.....	3:17	29.72	29.73	22.2
2:59	29.73	29.74	.....	....	.....	.....	.....

Cooling of calorimeters in eleven minutes; 0.13 degrees for R. and L. Mouth temperature 37.05 C. Volume of right hand in calorimeter, 487 c.c., of left hand 456 c.c. Water equivalent of calorimeters with contents, R., 3.520; L., 3.495.

As was to be expected from the fact that the inflammation, although unilateral, does not involve either hand, the blood-flow is approximately the same in the two hands (3.18 grams per 100 c.c. per minute for the right hand and 3.40 grams for the left hand, with room temperature 22.2 C.). So far as it goes the deficiency in the right hand as compared with the left, if the markedly subnormal flow for the age of the patient is determined in any important degree by reflex impulses from the inflamed area, may be due to the closer anatomical connection of the afferent paths from the right elbow with the efferent paths to the right than with those to the left hand. Another factor which probably contributed to the small flow is that the man came to the dispensary on a cold day without gloves and could not be kept for a sufficiently long interval before the examination. Also he was a teamster, accustomed to the exposure of the hands to cold, and there is some evidence that these

"cold-adapted" hands require an unusually long time to respond to our standard conditions of temperature.

It may be instructive to cite here a case in which one hand remained persistently swollen without any symptoms or history of inflammation.

Miss M. F., 34 years of age, 5 feet, 2 inches in height, a cook in a restaurant, was seen at the dispensary Feb. 16, 1911, on which day the blood-flow in the hands was measured. The left hand was permanently swollen to a moderate degree. The swelling came on about two years previously and had not varied to any extent. There was no pain or discomfort and no loss of power, but she felt the left hand to be colder than the right, especially in summer. There was a transverse scar on the radial side of the back of the wrist which she said was due to the lancing of a boil about the time the swelling came on. She did not connect the lancing of the boil with the swelling, to which, indeed, she paid no attention, coming to the dispensary not on her own account, but with a friend. Nevertheless on cross-examination she said she felt pretty sure that there was no swelling until after the boil had been lanced. On the day of examination both hands felt rather cold, and to the touch there was no difference between them. Pulse 110 (sitting). Hands in bath at 3:53 p. m., in calorimeters at 4:04 and out of calorimeters at 4:23. Mouth temperature 37.02 C.

TABLE 12.—CALORIMETER MEASUREMENTS IN CASE OF MISS F.

Time	Right	Left	Room Temp., C.	Time	Right	Left	
4:03	29.96	29.50	.....	4:15	30.11	29.71	.....
4:05	29.95	29.50	.....	4:16	30.12	29.72	.....
4:06	29.96	29.50	23.9	4:17	30.13	29.75	.....
4:07	29.96	29.52	.....	4:18	30.18	29.81	.....
4:08	29.97	29.53	.....	4:19	30.19	29.83	.....
4:09	29.99	29.56	.....	4:20	30.22	29.85	.....
4:10	30.01	29.60	.....	4:21	30.25	29.88	.....
4:11	30.04	29.62	.....	4:22	30.28	29.90	.....
4:12	30.06	29.65	.....	4:23	30.29	29.91	.....
4:13	30.07	29.68	.....	4:40	30.11	29.73	.....
4:14	30.09	29.70	24.1	.....	.....	.....	.....

Cooling of calorimeters in seventeen minutes, 0.18 degrees for R. and L. Volume of right hand in calorimeter, 355 c.c., of left hand 380 c.c.

The flow in the right hand comes out 4.81 grams per 100 c.c. per minute, in the left 4.78 grams (or, allowing for the probable amount of edema liquid, 5.4 grams), with room temperature at 24 C. The contrast with the cases of inflammation is striking. The flow in the affected hand is somewhat more copious than in the other, indicating that the cause of the edema is probably not obstruction to the venous return. In view of the coincidence between the appearance of the edema and the opening of the boil, it seems most natural to connect the swelling with injury to the radial nerve not as yet recovered from, since there is no indication of any infection of the lymphatics at that time. It is true that Simons<sup>10</sup>

10. Simons: Arch. f. Physiol., 1910, p. 559.

has stated that the radial nerve does not carry vasomotor fibers to the hand. But even if this be the case, and the evidence is by no means conclusive, this does not preclude the production of permanent vasomotor effects through an injury to a nerve. Infection of the nerve path at the seat of injury, as might occur if the nerve were divided through the boil, might delay regeneration. Thrombosis of veins or lymphatics might also be thought of, but with the free anastomosis it is difficult to see how this could cause permanent edema. The fact that it was in summer that she felt the left hand cold in comparison with the right indicates that the vasomotor response to external heat is less perfect than in the normal hand. A case of edema in the foot and leg probably connected with a traumatic injury will be referred to in another connection (Paper XI of this series, case of Charles H.).

In this connection may be mentioned experiments in which the flow was tested in a normal man (M. C.) after bandaging one hand so as to cause temporary anemia of the part, or after bandaging one forearm so as to cause temporary congestion of the hand. In both cases the bandage was removed just before the hands were put into the calorimeters.

M. C., a normal man 22 years old, height 5 feet, 10 inches, weight 146 pounds (stripped). April 17, 1911, an experiment was conducted to test the effect of bandaging the hand on the blood-flow after removal of the bandage. At 3:38 p. m. began bandaging the left hand from the finger tips upward with a rubber bandage (Martin's). Bandaging was completed at 3:39½ p. m., at which time both hands were immersed in the bath, the temperature of which was 30.2 C. At 3:50 p. m. the hands were put into the calorimeters, the bandage having been rapidly removed from the left hand while it was still kept in the bath. At 4:04 p. m. the hands were removed from the calorimeters. Volume of right hand in calorimeter 473 c.c., of left hand 448 c.c. Rectal temperature 36.9 C.

TABLE 13.—CALORIMETER MEASUREMENTS IN A NORMAL INDIVIDUAL AFTER THE HANDS HAD BEEN BANDAGED

Time	Right	Left	Room Temp., C.	Time	Right	Left	Room Temp., C.
3:48	29.99	30.04	23.8	3:59	30.31	30.44	24.4
3:51	30.02	30.08	.....	4:00	30.37	30.50	.....
3:52	30.06	30.15	.....	4:01	30.41	30.54	24.4
3:53	30.09	30.21	24.4	4:02	30.45	30.59	.....
3:54	30.12	30.23	.....	4:03	30.49	30.61	24.4
3:55	30.16	30.28	.....	4:04	30.52	30.64	.....
3:56	30.19	30.32	.....	4:04½	30.52	30.64	.....
3:57	30.22	30.35	.....	4:10½	30.47	30.60	24.2
3:58	30.28	30.41	.....	.....	.....	.....	.....

Here the flow per 100 c.c. per minute in the right hand works out at 6.25 grams and in the left hand at 7.04 grams for thirteen minutes in calorimeters. For the first 11 minutes of this period the calculated flows

are 6.26 grams per 100 c.c. per minute for the right hand and 7.45 grams for the left. The flow in the previously anemic hand is therefore increased to a moderate degree. Probably the increase is somewhat greater than it appears to be from these figures, since the flow per 100 c.c. per minute in M. C. is nearly always somewhat less for the left than for the right hand. In both hands the flow is less than usual for this man, as the hands had been long used for blood-flow observations on the same afternoon, and this causes vasoconstriction.

M. C., May 4, 1911. Experiment on the effect of bandaging the forearm on the flow in the hand after removal of the bandage. The left forearm was bandaged from above downward with a Martin's bandage. The bandaging was begun immediately below the bend of the elbow and carried down nearly to the upper pencil mark on the wrist, the arm being all the time in the dependent position. The hand was well filled with blood before the application of the bandage was begun and the bandage was tight enough to retain the venous blood without greatly affecting the arterial flow.

At 2:37 p. m. the hands were put into the bath and at 2:47 into the calorimeters, the bandage having been rapidly removed from the left forearm just before the hands were taken out of the bath. The hands were taken out of the calorimeters at 3:06 p. m. Pulse (sitting) 90. Cooling of calorimeters in nine minutes: R., 0.115 degrees; L., 0.11 degrees. Volume of right hand in calorimeter 486 c.c., of left 470 c.c. Water equivalent of calorimeters with contents, R., 3,484, L., 3,471. Rectal temperature 37.2 C.

TABLE 14.—CALORIMETER MEASUREMENTS AFTER BANDAGING THE FOREARM

Time	Right	Left	Notes	Time	Right	Left	Notes
2:45½	29.54	29.63		2:58	30.58	30.47	Room 23.2.
2:48	29.61	29.66	He feels left hand warmer than right.	2:59	30.67	30.54	
2:49	29.72	29.75		3:00	30.74	30.61	Left now feels water cool the whole time of stirring like right, but not as distinctly as right.
2:50	29.81	29.84					
2:51	29.90	29.92	Right hand feels water cool when stirred, but not left. Left feels tired.				
2:52	29.99	29.98		3:01	30.83	30.69	
2:53	30.11	30.07	Room 23.3.	3:02	30.93	30.76	Now feels no difference in warmth of the two hands, but left still a little tired.
2:54	30.17	30.13					
2:55	30.29	30.25	Left still feels warmer than right.				
2:56	30.41	30.34		3:03	31.01	30.83	
2:57	30.47	30.40	Left feels water only when stirring is begun.	3:04	31.09	30.90	Room 23.2.
				3:05	31.17	30.94	
				3:06	31.235	31.01	The hands now feel the same.
				3:15	31.12	30.90	Room 22.8.

For the first nine minutes in the calorimeters the flow comes out 12.86 grams per 100 c.c. per minute for the right hand, and 11.61 grams for the left. For the second nine minutes the flows are 13.31 grams, and 10.96 grams for the right and left hands, respectively; for the whole



eighteen minutes, 13.12 grams and 11.26 grams. The disproportion between the flows in the two hands cannot be due to the deeper parts of the left hand having more nearly acquired the temperature of the bath owing to the impeded circulation and being then heated up by the blood during the first part of the period of immersion in the calorimeters. For the disproportion is greater in the second than in the first nine minutes. The combined flow in the two hands remains practically constant throughout.

In the experiment of May 24, 1911, on the same man, the effect of moderate compression of the veins by a rubber band kept on one forearm during the observations is illustrated. Although the diminution in the flow is distinct, it is far less than might have been expected from the swelling of the veins in the hands. The reason is that the venous pressure quickly rises to the point at which blood again passes freely heart-wards under the band. Evidence will be brought forward in the next paper (Paper X) that some degree of venous engorgement in certain cardiac lesions in which the beat of the heart is still strong and orderly, the myocardium not having seriously deteriorated, may even be associated with an increased hand flow, the rise of venous pressure, which is general, not local as in the experiment under discussion, being more than compensated by the concomitant rise in the arterial pressure and the widening of the vascular path.

M. C., May 24, 1911. Pulse (sitting) 87. Hands in bath at 2:29 p. m., in calorimeters at 2:39½, out of calorimeters at 3:10. Mouth temperature 36.7 C. At 2:48½ put band on right forearm about 8 cm. above the upper mark. The band was made of rubber tubing. It compressed the veins without affecting the radial pulse so far as could be felt by the finger. After it was put on the subject said his right hand felt "heavy," and later on had a kind of burning sensation. Band taken off at 3:00½ p. m. Cooling of calorimeters: R., 0.95 degrees in 9 minutes; L., 0.10 degrees in nine and one-half minutes. Volume of right hand in calorimeter, 470 c.c.; of left hand, 457 c.c. Rectal temperature 37.3 C.

For five minutes before the application of the band the flow works out at 17.98 grams per 100 c.c. per minute for the right hand, and 16.65 grams for the left, with the high room temperature of 26.9 C. For the first five minutes with the band on the right arm the flows were, respectively, 15.55 grams per 100 c.c. per minute for the right, and 15.82 grams for the left hand. For the last six minutes of application of the band the flows were 13.56 grams and 13.93 grams for the right and left hands, respectively. The original preponderance of flow in the right hand has therefore been changed by the obstruction to the venous return from the right hand to a preponderance in favor of the left. That this is not due to some accidental (vasomotor?) effect in the course of the experiment is shown by the restoration of the preponderance of the right hand when the band is removed, the flow for nine minutes coming out at 15.20 grams per 100 c.c. per minute for the right, and 14.84 grams for the left hand.

The changes in the flow in the left hand suggest that the interference with the flow in the right (local asphyxia) may have caused some compensatory vasoconstriction elsewhere, especially in the left hand, as a means of raising the arterial pressure and thus helping to overcome the obstruction.

TABLE 15.—CALORIMETER MEASUREMENTS WITH RUBBER BAND ON ARM

Time	Right	Left	Room Temp., C.	Time	Right	Left	Room Temp., C.
2:39	30.29	30.33	.....	2:57	32.065	32.03	.....
2:41	30.48	30.51	26.5	2:58	32.11	32.10	26.4
2:42	30.62	30.64	.....	2:59	32.20	32.16	.....
2:43	30.76	30.78	27.1	3:00	32.26	32.225	.....
2:44	30.88	30.90	26.9	3:01	32.30	32.295	26.8
2:45	31.02	31.02	.....	3:02	32.37	32.335	.....
2:46	31.125	31.105	26.6	3:03	32.43	32.405	.....
2:47	31.22	31.205	.....	3:04	32.495	32.46	.....
2:48	31.34	31.30	.....	3:05	32.58	32.535	26.5
2:49	31.41	31.37	.....	3:06	32.65	32.61	.....
2:50	31.505	31.45	.....	3:07	32.72	32.68	.....
2:51	31.595	31.535	.....	3:08	32.785	32.735	26.9
2:52	31.68	31.63	.....	3:09	32.85	32.81	.....
2:53	31.775	31.73	26.6	3:10	32.905	32.87	.....
2:54	31.85	31.81	.....	3:19	32.81	.....	.....
2:55	31.92	31.89	.....	3:19 <sup>14</sup>	.....	32.77	26.5
2:56	31.995	31.95	.....	....	.....	.....	.....

Certain cases in which congenital or at least very long standing anatomical defects existed in one hand without markedly affecting its functional power were examined. Here, as was to be expected, no great difference in the flow per 100 c.c. of hand was made out.

Helen P., a girl 21 years old, height 4 feet 11 inches, well nourished. She works with a sewing machine in a factory. She said she had rheumatism at 6 months, and St. Vitus' dance two years previous to examination. She came to the dispensary for "nervousness." She has no symptoms of chorea, but is afraid she is going to have it again. Menstruation is disturbed, her period falling too late for the past two or three months, but there is no more than the usual amount of discomfort and the discharge is not altered in quantity. She has suffered much from headache the past two or three months. Her left wrist is deformed (subluxation anteriorly), the styloid process of the ulna being very prominent. The left hand is considerably smaller and the left ulna and radius shorter than the right. This has been true for a long time, perhaps from birth. The left hand is normally formed and she uses it in all ways in which a normal hand would be used, only it is not so strong as the right hand. The left anterior limb is shorter than the right and the girth of the forearm is distinctly less to the eye. The left hand does not get cold any more than the right and does not require to be better protected in winter. The pulse at the wrist is of fair volume to the finger. The blood-flow was examined July 20, 1911, the first day she came to the dispensary. Pulse (sitting) 88. Hands in bath at 2:21 p. m.,

in calorimeters at 2:45½ p. m. The lower mark on the left wrist had to be drawn through the middle instead of at the lower border of the styloid process of the ulna, as the hand was bent somewhat to the side, and the whole hand could not otherwise have been included in the calorimeter. Mouth temperature 37.6 C.

With the rather high room temperature of 26 C. the flow in the right hand was 9.08 grams per 100 c.c. of hand per minute, in the left 8.81 grams. The vasomotor reflexes from the right to the left hand were less marked than those normally observed, but in the normal direction.

TABLE 16.—CALORIMETER MEASUREMENTS IN CASE OF HELEN P.

Time	Right	Left	Notes	Time	Left	Notes
2:45	30.495	30.45	Room 26.0 C.	3:01	30.89	At 3:04 put right hand in water at 43 C.
2:47	30.575	30.47		3:02	30.93	
2:48	30.60	30.51		3:03	30.96	
2:49	30.64	30.54		3:04	30.97	
2:50	30.69	30.60				
2:51	30.73	30.63	Room 26.2 C.	3:05	30.99	Room 26.2 C.
2:52	30.775	30.65		3:06	31.005	
2:53	30.80	30.69		3:07	31.025	
2:54	30.85	30.72		3:08	31.04	
2:55	30.89	30.74		3:09	31.08	
2:56	30.92	30.765	At 2:56 put right hand in water at 8.3 C.	3:10	31.11	Room 26.1 C. At 3:12 hand out of calorimeters. At 3:17 right is at 30.77 C.
				3:11	31.135	
				3:12	31.155	
2:57	.....	30.80	She feels it very cold and complains of it. Still complains of the cold water, though it is now more tolerable.			
2:58	.....	30.825		3:17	31.12	
2:59	.....	30.835				
3:00	.....	30.87				

Cooling of calorimeters: R., 0.15 degrees in twenty-one minutes; L., 0.035 degrees in five minutes. Volume of right hand, 282 c.c., of left hand, 230 c.c.

Max II., aged 46, with spastic paralysis of the left wrist (birth palsy), showed a flow in the left hand but little less than that in the right (right hand 10.71 grams per 100 c.c. per minute, left hand 10.2 grams with room temperature 20.5 C.). These flows are perfectly normal. He uses the left hand largely in his work, that of a telegraph operator. It was obviously well supplied with blood. The protocol of the case has already been published.<sup>10</sup>

Marie G., 15 years old, congenitally deficient in intellect and brought on this account to the dispensary by her mother. July 20, 1911. She became violent at times and would bite her mother. On admission she was in good humor. She could read books and newspapers and did so regularly. She played the piano and easily picked up a melody which she heard. She could also write, but she was quite defective in certain points; for example, she did not understand the

10. Proc. Soc. Exper. Biol. and Med., 1910, viii, 43.

time of day. She did not walk until she was 3 years old. She had menstruated regularly for about two years prior to being seen. Her left hand was congenitally defective, the thumb being wanting; otherwise it was perfectly formed, though small. The left arm both upper and lower, was much smaller in girth than the right, and the elbow joint appeared very prominent between the two ill-developed segments. The left hand easily became cold in winter. She used it quite freely, but not so much as the right. The whole left anterior extremity was somewhat shorter than the right. The mother said the child's left leg was also affected to some extent, but this was not now noticeable in her walk. She was well grown and looked well nourished. The blood-flow in the hands was examined July 20, 1911. Hands in bath at 3:24 p. m., in calorimeters at 3:26. Mouth temperature 37.35 C. At 3:50 p. m. right hand put into water at 42.8 C. Cooling of calorimeters: R., 0.12 degrees in twenty-two minutes; L., 0.14 degrees in nineteen minutes. Pulse (sitting) 88. Volume of right hand in calorimeter, 260 c.c.; of left hand, 170 c.c.

TABLE 17.—CALORIMETRIC MEASUREMENTS OF MARIE G.

Time	Right	Left	Room Temp., C.	Time	Left	Notes
3:35¼	30.13	30.20	.....	3:56	30.435	At 4:03 right hand put in water at 13 C. She says she feels it very cold.
3:37	30.12	30.17	26.2	3:57	30.47	
3:38	30.125	30.17	.....	3:58	30.50	
3:39	30.145	30.19	.....	3:59	30.525	
3:40	30.16	30.18	.....	4:00	30.535	
3:41	30.185	30.21	.....	4:01	30.54	Room at 26.3 C.
3:42	30.20	30.22	26.5	4:02	30.56	
3:43	30.225	30.23	.....	4:03	30.565	
3:44	30.25	30.23	.....	4:04	30.59	
3:45	30.28	30.24	.....	4:05	30.60	
3:47	30.31	30.265	.....	4:06	30.61	Hand removed from calorimeter at 4:11. Temp. of R. at 4:12, 30.28.
3:48	30.335	30.30	.....	4:07	30.62	
3:49	30.38	30.325	.....	4:08	30.625	
3:50	30.40	30.335	.....	4:09	30.635	
3:51	.....	30.35	26.25	4:10	30.64	
3:52	.....	30.355	.....	4:11	30.65	
3:53	.....	30.36	.....	4:12	.....	
3:54	.....	30.38	.....	4:30	30.51	
3:55	.....	30.41	.....	.....	.....	

With average room temperature 26.3 C. the flow in the right hand was 5.96 grams per 100 c.c. per minute, and in the left 6.6 grams per 100 c.c. per minute. The flow in the defective hand is thus somewhat greater than in the normal one, in contrast to the condition in a case of infantile paralysis in N. M., a boy 9½ years old, whose left hand was much atrophied and functionally of little use. In his right hand the flow was 15.0 grams per 100 c.c. of hand per minute against 7.6 grams in the left hand with room temperature 23.4 C. The protocol of N. M. has already been published.<sup>10</sup>



It has been mentioned in a previous paper<sup>11</sup> that the flow in the distal half of the hand is greater than in the hand as a whole, doubtless because of the greater proportion of skin in the former. A case (John B.) in which some of the fingers of the left hand had been amputated, gave the opportunity of comparing two hands, one of which had suffered a considerable loss in its distal half. The blood-flow for the last ten minutes in the calorimeters was 7.20 grams per 100 c.c. per minute for the right and 5.34 grams for the left hand, a considerable deficiency in the hand which had lost fingers. The fact that the man had a right-sided pleurisy with effusion probably does not interfere with this conclusion, but, if anything, strengthens it. For it will be shown in a subsequent paper that this condition is usually associated with a diminished hand flow on the side of the lesion and that the difference may persist after the fluid has been removed.

John B., a longshoreman aged 51, admitted to City Hospital April 28, 1913, suffering from pulmonary tuberculosis, and pleurisy with effusion on the right side. He had a severe cold in the winter and in a short time the visiting nurse said he had tuberculosis. He was a well developed, well nourished man, weighing about 185 pounds, height about 5 feet, 11 inches. No cyanosis. Dyspnea present with physical signs of liquid in right pleural cavity. The heart presents nothing abnormal. Pulse full and regular. The sputum contains tubercle bacilli. May 4, at 4 p. m., 1,500 c.c. of straw-colored fluid was removed from the right thorax. Maximum temperature following admission 100.4 F. at 4 p. m., May 5. The whole middle finger and the second and third phalanges of the index finger of the left hand had been amputated for an injury. The blood-flow in the hands was measured May 6, 1913. Pulse (sitting) 100. Hands in bath at 2:46 p. m., in calorimeters at 2:57 and out of calorimeters at 3:15 p. m.

TABLE 18.—CALORIMETER MEASUREMENTS IN CASE OF JOHN B.

Time	Right	Left	Room Temp., C.	Time	Right	Left	Room Temp., C.
2:56½	31.32	31.27	.....	3:07	31.68	31.455	.....
2:58	31.315	31.25	24.3	3:08	31.72	31.485	.....
2:59	31.33	31.265	.....	3:09	31.78	31.51	.....
3:00	31.37	31.28	.....	3:10	31.81	31.54	24.6
3:01	31.40	31.29	24.4	3:11	31.86	31.56	.....
3:02	31.43	31.315	.....	3:12	31.90	31.59	.....
3:03	31.47	31.34	24.35	3:13	31.94	31.64	24.7
3:04	31.52	31.36	.....	3:14	32.01	31.67	.....
3:05	31.57	31.39	.....	3:15	32.06	31.72	.....
3:06	31.62	31.44	24.2	3:32	31.84	31.53	.....

Cooling of calorimeters in seventeen minutes: R., 0.22 degrees; L., 0.19 degrees. Volume of right hand in calorimeter, 564 c.c., of left hand, 515 c.c. Rectal temperature 38.32 C. Water equivalent of calorimeters and contents, R., 3.546; L., 3.507. Blood-pressure, left arm, systolic 106; 93 (sound began to diminish but not suddenly); 77 (sound gone).

11. Heart, 1911, iii, 40.

The last case to be mentioned is that of Mrs. K., a woman 68 years old in whom Dr. Carl A. Hamann had ligated the innominate and right common carotid arteries for aneurysm of the subclavian.

Mrs. K., aged 68. The innominate and right common carotid arteries were tied at Charity Hospital by Dr. Carl Hamann on Feb. 26, 1913, for aneurysm of the subclavian artery. Wiring of the sac had previously been tried without effect. March 20, 1913, the blood-flow was compared in the two hands. No pulse could be felt in the right wrist or over the right brachial. She said the right hand got cold if left uncovered with bed-clothes. When hung down the veins filled well, as well as in the left hand. She said she felt the blood going down into the right hand when she hung it down. On being emptied by stripping, the veins on the back of the right hand filled from below slowly. The right hand could execute all movements, but was weak. She spoke of the loss of power in it. There was some paresthesia (pins and needles and numbness) in the right hand, but this she had before the operation, and it had not been intensified since. The nails on the right hand were getting clubbed. She noticed that they were getting "like those of a man." The change in the nails was present before the operation, but was now more marked. First examination of the blood-flow in the hands March 20, 1913. Hands in bath at 1:50½ p. m., in calorimeters at 2:00 and out of calorimeters at 2:13. Mouth temperature 37.0.

TABLE 19.—CALORIMETRIC MEASUREMENTS IN CASE OF MRS. K.

Time	Right	Left	Room Temp., C.	Time	Right	Left	Room Temp., C.
1:59	30.78	30.65	26.2	2:08	30.675	30.70	.....
2:02	30.68	30.62	.....	2:09	30.67	30.72	26.7
2:03	30.675	30.64	26.5	2:10	30.67	30.75	.....
2:04	30.67	30.65	.....	2:11	30.675	30.78	.....
2:05	30.67	30.66	.....	2:12	30.675	30.795	.....
2:06	30.67	30.67	26.7	2:13	30.685	30.82	.....
2:07	30.675	30.69	.....	2:23	30.62	30.74	.....

Cooling of calorimeters in ten minutes: R., 0.07 degrees; L., 0.08 degrees. Volume of right hand, 378 c.c.; of left hand 360 c.c. Repeated the volume measurement with the same results. The right hand was not swollen. Pulse (sitting) 124. Water equivalent of calorimeters with contents. R., 3.397; L., 3.383.

Second examination March 21, 1913. The weather was decidedly colder than on the previous day. Pulse (sitting) 100. Mouth temperature 36.5 C. Hands in bath at 2:52 p. m.; in calorimeters at 3:02 p. m., and out of calorimeters at 3:25 p. m. At 3:18 the left hand was put into water at 43 C.

The blood-flows at the first examination work out at 1.50 grams per 100 c.c. of hand per minute for the right hand and 5.32 grams for the left hand (for the last five minutes in the calorimeters) with room temperature 26.7 C. The ratio between the flows in the two hands is 1:3.54. At the second examination the flow was 1.83 grams per 100 c.c. per minute in the right hand, and 6.38 grams in the left, with room temperature 22.7 C., the ratio being 1:3.48, almost identically the same ratio as at the first examination. The increased flow in both hands is

therefore due to a central cause (better heart action, with a slower pulse?). Immersion of the left hand in warm water caused practically no change in the flow in the right. It is obvious that vasodilatation in the right hand could not be expected to materially affect the flow through the narrow and difficult collateral path. The block does not lie in the peripheral portions of the vascular path and cannot be removed by dilatation of peripheral areas.

TABLE 20.—SECOND CALOMETRIC TEST IN CASE OF MRS. K.

Time	Right	Left	Room Temp., C.	Time	Right	Left	Room Temp., C.
3:01	31.00	30.99	.....	3:15	30.925	31.08	22.7
3:03	30.93	30.92	22.7	3:16	30.92	31.10	.....
3:04	30.93	30.92	.....	3:17	30.925	31.125	22.7
3:05	30.93	30.925	22.7	3:18	30.925	31.15	.....
3:06	30.93	30.925	.....	3:19	30.93	.....	22.9
3:07	30.93	30.935	22.75	3:20	30.93	.....	.....
3:08	30.93	30.95	.....	3:21	30.93	.....	22.85
3:09	30.93	30.97	22.75	3:22	30.93	.....	.....
3:10	30.93	30.985	.....	3:23	30.925	.....	22.9
3:11	30.925	30.995	22.7	3:24	30.925	.....	.....
3:12	30.925	31.01	.....	3:25	30.925	.....	22.8
3:13	30.925	31.03	22.6	3:40	30.77	30.89	.....
3:14	30.925	31.06	.....	....	.....	.....	.....

Cooling of calorimeters: R., 0.155 degrees in fifteen minutes; L., 0.26 degrees in twenty-two minutes. Volume of right hand in calorimeter 369 c.c., of left hand 342 c.c. Water equivalent of calorimeters and contents, R., 3,390; L., 3,369. Blood-pressure in left arm, systolic 136; 69 (sound gone).

July 9, 1913, a third blood-flow examination was made. The patient was much improved and was doing all her own housework. The grip of the right hand was fair, though weaker than the left. She had gained considerably in weight and her arms and hands were distinctly more plump. There was no pulse in the radial. The veins on the back of the hand filled from below when emptied and much more quickly than at the last examination. She suffered a good deal from pain in the arm and shoulder. A report from Dr. Hamann, who saw her on June 24, 1913, is as follows:

"It is altogether likely that there is pressure on the brachial plexus by the end of the clavicle (a portion of the clavicle was resected at the operation), the new formed bone (which can be distinctly felt) and the cicatrix, and removal of more of the clavicle would most likely cure or relieve her. The aneurismal sac has been consolidated and but little trace of it can be felt. There is no evidence of any intrathoracic aneurism."

The results of the third blood-flow examination are entirely favorable to this view, for they show that any disability in the right hand was not now due to deficient circulation, and that the surgeon need not be deterred from operating, if an operation seemed desirable, by the fear that the symptoms might be dependent on a poor blood-flow.

The flow in the right hand came out 8.26 grams per 100 c.c. per minute and in the left 10.69 grams, for a period of twenty-three minutes, with average room temperature 26.2 C., almost the same temperature as at the first examination. The flow in both hands was considerably increased. It must be remembered that not only was the patient much stronger than when examined before, but that instead of being brought from her bed for the observations, she had come several miles on the street car. Her pulse was strong and at the rate of 104 per minute. Both hands were larger than before (right 424 c.c., left 385 c.c.).

The fact that the collateral circulation was much freer than before is particularly shown in the increase of the ratio of the flow in the two hands from 1:3.5 to 1:1.3. It is fully as well brought out by the result of the examination of the vasomotor reflexes. Immersion of the left hand in cold water caused a great diminution in the flow in the right — for the first two minutes to 2.41 grams per 100 c.c. per minute. For the next five minutes the flow was only 6.19 grams per 100 c.c. per minute, and then began to increase abruptly. Reflex vasoconstriction, therefore, was capable of markedly altering the flow in the right hand. The relative resistance of the smaller vessels of the anterior limb and of the collateral paths leading to it has accordingly changed, and the resistance in the latter does not now make up such a large proportion of the total resistance as before.

In early brachial neuritis it is usual to see a greater flow in the hand of the affected side than in that of the sound side (partial loss of vasoconstrictor tone) and the crossed vasomotor reflex to cold is intense. It is possible that in this patient part of the increase of the flow in the right hand since the last examination is due to this factor, and the good reflex vasoconstriction agrees with this.

#### SUMMARY

The observations in this paper were partly undertaken as tests of the technic of the method and do not lend themselves to a complete summary.

1. In a man with healing burns on the hands the apparently great vascularity of the new tissue did not correspond to an abnormally great flow. Certain phenomena interpreted as due to a peculiar vasomotor instability of the new vessels were observed in testing the vasomotor reflexes.

2. A relatively good flow in the feet was seen in a man with great and persistent edema of both legs in whom a diagnosis of Hodgkin's disease was made. This was interpreted as indicating obstruction rather on the lymph path than on the venous path.

3. Three cases of unilateral inflammation in the hand, one bacterial and two non-bacterial (gout and a sprain) were compared. The flow in



TABLE 21.—SUMMARY OF

Case	Age	Date	Pulse- Rate	Blood- Pressure	Temperature of				Volume of Part in c.c.	
					Room	Art. Blood	Calorimeters		Right	Left
							Right	Left		
Steve S. ....	21	4/ 5/12	80	.....	25.0	36.8	30.14	30.06	328	340
					24.8	.....	30.59	.....	.....	.....
					24.8	.....	30.86	.....	.....	.....
Frank P. ...	42	8/ 1/12	96	114.80	23.2	37.45	30.44	30.40	476	476
Charles B....	47	11/27/12	108	155.95	22.3	36.75	30.71	30.73	478	477
		11/29/12	98	.....	22.5	36.3	31.33	31.48	1,313	1,313
					23.0	36.4	30.82	30.78	480	462
Charles W..	42	12/18/12	92	.....	23.9	37.3	31.52	31.01	1,203	1,103
					23.6	37.4	31.61	31.57	2,422	2,221
					19.1	37.0	29.56	29.56	1,272	1,303
Philip D....	47	12/20/10	68	.....	23.8	37.8	30.59	29.87	580	500
Charles C...	36	2/27/11	116	.....	23.6	.....	37.42	.....	.....	.....
					23.6	.....	31.97	.....	.....	.....
					24.3	37.1	30.57	30.29	456	404
Harry S....	50	4/ 3/12	.....	.....	24.5	.....	30.78	.....	.....	.....
					24.3	.....	30.96	.....	.....	.....
					24.1	.....	31.12	.....	.....	.....
		7/ 9/12	100	111.86	24.0	.....	31.24	.....	.....	.....
					29.7	37.45	31.97	31.70	530	410
					29.7	.....	32.56	.....	.....	.....
Martin B...	66	1/19/11	.....	151.0	29.8	.....	33.00	.....	.....	.....
					23.2	37.38	29.50	29.59	490	497
					22.2	37.05	29.78	29.80	487	456
George S...	37	2/ 2/11	92	.....	24.0	37.0	30.13	29.72	355	380
Miss M. F...	34	2/16/11	110	.....	24.4	36.4	30.27	30.36	473	448
M. C. ....	22	4/17/11	.....	.....	23.3	36.7	30.04	30.03	486	470
		5/ 8/11	.....	.....	23.2	.....	30.85	30.71	.....	.....
		5/24/11	.....	.....	26.9	36.8	31.05	31.04	470	457
					26.6	.....	31.63	31.59	.....	.....
					26.5	.....	32.06	32.02	.....	.....
					26.7	.....	32.60	32.58	.....	.....
Helen P. ...	21	7/20/11	88	.....	26.0	37.6	30.76	30.64	282	230
					26.1	.....	.....	30.87	.....	.....
					26.1	.....	.....	31.06	.....	.....
Marie G. ...	15	7/20/11	88	.....	26.3	37.3	30.28	30.26	260	170
					26.3	.....	.....	30.45	.....	.....
					26.3	.....	.....	30.6	.....	.....
John B. ...	51	5/ 6/13	100	106(93)77	24.4	37.8	31.82	31.56	564	515
		3/20/13	124	.....	26.7	37.0	30.68	30.74	378	360
		3/21/13	100	136.69	26.6	.....	30.68	30.72	.....	.....
Mrs. K. ....	68	7/ 9/13	104	.....	22.7	36.5	30.93	31.05	369	342
					26.2	37.0	31.89	32.03	424	385
					26.2	.....	32.39	.....	.....	.....
					26.4	.....	32.50	.....	.....	.....

\* Allowing 300 c.c. for edema fluid in each foot the flow would be 3.04 for the right and 3.24

† Allowing for the probable amount of edema fluid these flows would be 13.05, 12.20, and

‡ Allowing for edema fluid these flows would be 7.47, 5.06, 10.73, 6.90, and 4.16.

§ Allowing for edema fluid these flows would be 13.27, 8.47, and 12.82.

¶ Allowing for edema fluid, 5.4.

THE AUTHOR'S CASES

Heat Given Off in Gram-Calories			Blood-Flow in Gm. Per Min.		Flow per 100 c.c. of Part per Min.		Notes
Right	Left	In Min.	Right	Left	Right	Left	
1.242	1,010	9	23.09	18.50	7.04	5.44	Burned hands; right worse.
2.283	.....	12	34.04	.....	10.38	.....	Left hand in water at 40 C.
621	.....	8	14.52	.....	4.42	.....	Left hand in water at 10 C.
2.537	2,848	18	22.34	25.15	4.69	5.31	Tumor of right forearm.
506	556	18	5.15	5.70	1.08	1.19	Hands } Hodgkin's disease
3.030	3,139	22	20.79	32.89	2.34*	2.50†	Feet } with edema of
1.159	1,282	17	13.57	14.90	2.82	3.22	Hands } legs and feet.
1.183	974	10	22.76	17.20	1.89	1.56	Feet } Trophic disturbance
486	470	9	51.62	47.03	10.62	10.00	Hands } in left leg.
385	375	7	27.14	27.80	7.05	7.41	Right arm shorter than left.
4.041	1,580	9	69.19	24.61	11.93‡	4.92	Infected right hand.
2.600	.....	7	64.67	.....	11.15‡	.....	Left hand in water at 9.2 C.
1.845	.....	5	70.33	.....	12.12‡	.....	Left hand in water at 4.3 C.
1.661	684	9	31.40	12.60	6.88‡	3.07	Gout; right hand.
242	.....	2	21.27	.....	4.66‡	.....	Left hand in water at 4.3 C.
1.245	.....	5	45.06	.....	9.88‡	.....	Left hand still in water at 4.3 C.
312	.....	2	28.98	.....	6.35‡	.....	Left hand in water at 8 C.
554	.....	6	17.50	.....	3.84‡	.....	Left hand still in water at 8 C.
3.096	1,899	11	57.06	33.36	10.76§	8.13	Right hand worse than before.
1.443	.....	9	36.43	.....	6.87§	.....	Left hand in water at 8.3 C.
1.988	.....	9	55.15	.....	10.40§	.....	Left hand in water at 42.8 C.
700	2,180	12	8.20	25.87	1.7	5.20	Sprain of left hand.
710	710	7	15.52	15.54	3.18	3.40	Inflamed right elbow.
1.690	1,903	16	17.08	18.16	4.81	4.78†	Edema of left hand.
2.118	2,244	13	29.54	31.76	6.25	7.09	After bandaging left hand.
3.380	2,950	9	62.48	54.60	12.86	11.61	After bandaging left arm.
3.066	2,499	9	66.70	51.53	13.31	10.96	Second period.
2.187	1,972	5	84.51	76.09	17.98	16.65	
1.701	1,695	5	73.11	72.31	15.55	15.82	After putting band on right arm.
1.631	1,663	6	63.73	63.67	13.56	13.93	Band still on right arm.
2.430	2,348	9	71.43	67.82	15.20	14.84	After removing band.
1.262	1,016	8	25.62	20.28	9.08	8.81	Left hand smaller than right.
.....	852	8	.....	17.59	.....	7.51	Right hand in water at 8.3 C.
.....	787	8	.....	16.71	.....	7.26	Right hand in water at 4.3 C.
979	711	10	15.5	11.22	5.96	6.6	Congenital defect of left hand.
.....	1,034	13	.....	12.90	.....	7.59	Right hand in water at 42.8 C.
.....	388	8	.....	8.04	.....	4.73	Right hand in water at 13 C.
2.188	1,547	10	40.65	27.54	7.20	5.34	Left hand partially amputated.
162	540	5	5.69	19.16	1.50	5.32	After ligation of innominate.
278	967	11	4.44	15.23	1.12	5.32	Last five minutes in calorimeter.
373	1,179	11	6.76	21.85	1.83	4.23	Whole period in calorimeters.
3.708	4,236	23	35.05	41.17	8.26	6.38	
85	.....	2	10.24	.....	2.41	10.69	Left hand in water at 10.5 C.
532	.....	2	26.27	.....	6.19	....	Left hand still in water at 10.5 C.

for the left.  
13.27

the infected hand was increased, while in the normal hand it was diminished. In the case of gout, while the flow in the inflamed hand was greater than in the normal hand the difference was much less than in the case of infection. The contralateral vasoconstrictor reflexes from the normal to the inflamed hand were very slight in the case of the infected hand; of normal intensity in the case of gout. It is suggested that this indicates the existence of a relative vasoconstrictor block in bacterial inflammation in the interests of a permanently large flow of blood to combat the infection.

4. Temporary anemia of the hand (produced by bandaging) was followed by a moderate increase in the flow in a normal man.

5. In cases of congenital differences or differences originating in early life between the two hands, the blood-flow in the defective hand corresponded to its functional condition, being of normal magnitude when the power of the hand was good.

6. In a case in which some of the fingers of one hand had been lost by amputation, the flow per unit of volume was distinctly less in the defective than in the intact hand. This is mainly because the surface of the fingers is, in proportion to their bulk, so much greater than that of the rest of the hand. It is for the same reason that the flow per unit of volume is much greater in the distal half of the normal hand than in the hand as a whole.

7. In a case in which the innominate and the right common carotid arteries had been ligated one month previously, the flow in the right hand was two-sevenths of that in the left, although no pulse could be felt in the radial. Sixteen weeks later the ratio had risen to 1:1.3, the flow in the left hand being normal in amount.

## BLOOD TRANSFUSION AND REGENERATION IN PERNICIOUS ANEMIA \*

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A feature of much interest in the observation of cases of pernicious anemia is the behavior of the reticulated cells. In six cases of pernicious anemia, four of them treated by transfusion, which are here reported, this element of the blood-picture was studied with special care, and from these as well as from earlier cases of transfusion done in St. Luke's Hospital, certain conclusions are drawn in regard to the value of vital staining in cases of severe anemia, and concerning the subject of transfusion in general.

Ever since the discovery of the fact that by treating fresh, unfixed blood with solutions of various dyes, such as brilliant cresyl blue, polychrome methylene blue, neutral red, pyronin methylene green, etc., a curious and otherwise invisible structure could be demonstrated in the red cells, there has been much speculation as to its significance. Ehrlich, Pappenheim and Horsley were among the first to describe the phenomenon, but nearly all the subsequent studies have come from French and Italian laboratories (Hertz,<sup>1</sup> Ferrata and Boselli<sup>2</sup>). In suitably treated preparations this so-called granulo-filamentous or reticulo-filamentous substance appears in the form of coarse granular particles which are sometimes discrete, but more often occur in threads which frequently are woven into skeins or wreaths of great complexity and fill a considerable portion of the cells. In the blood of infants these reticulations are found in from 5 to 10 per cent. of the erythrocytes and in normal adult blood in from 0.5 to 2 per cent. In severe anemias, however, their number is much increased, running as high as 18 to 20 per cent., while in hemolytic jaundice, which is the condition par excellence for their prevalence, they may occur in still greater proportions.

Without taking the space at this time to enter into a discussion of the arguments pro and con of the various writers who have studied the matter, we may summarize briefly the views to which our own observations and a consideration of the literature have led us, by saying that the granulo-filamentous substance is not derived from the nucleus, is

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\* Submitted for publication July 21, 1913.

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1. Hertz: *Folia haematol.*, Arch., 1910, x, 419.

2. Ferrata and Boselli: *Folia haematol.*, Arch., 1910, x, 451.



different from polychromatophilia and from the basophilic stippling seen in fixed preparations stained with panchromatic dyes, is not a preformed structure, but is a precipitation product of the stain, and is an evidence of youth in the cells and not of degeneration. In conditions in which a severe drain on the erythrocytes is being sustained by a well-functioning bone-marrow, large numbers of reticulated cells are found, whereas in aplastic cases they may be diminished almost to the point of absence, that is, in a manner somewhat comparable to the behavior of the erythroblasts the reticulated cells afford a direct insight into the hematopoietic activities of the bone-marrow. For clinical purposes they form a more convenient measure of this function than do the nucleated cells, as their percentage relations to the erythrocytes can be more easily and accurately determined, and their enumeration is to be urged as a part of the study of the blood in all cases of severe anemia. The relationship between the presence of the reticulated cells in the peripheral blood and the hemopoietic activity of the marrow was well illustrated in the case of a young man suffering from purpura hemorrhagica seen since the above was written. In spite of three transfusions of about 700 c.c., each done by direct intravenous injection, the red cells rapidly became reduced to 1,500,000, hemoglobin 25 per cent., while the leukocytes were 2,800, polymorphs 12 per cent. and lymphocytes 88 per cent., death resulting within two weeks of the onset of the symptoms. A highly significant feature was the total absence of reticulated cells, showing the complete inability of the bone-marrow to respond to stimulation.

The following technic for vital staining may be used:

#### TECHNIC FOR VITAL STAINING

Saturate 0.85 per cent. salt solution with brilliant cresyl blue. Filter this through a double paper to take out the excess of dye substance and to prevent precipitation on the slide. It is better to centrifugalize the stain before using it, in order to be sure that no undissolved particles remain in suspension. If any precipitate is present in the stain it will later be thrown down on centrifugalizing the mixed blood and stain and will cause confusion in counting the reticulated cells.

The following should be freshly prepared before use:

Saturated solution of brilliant cresyl blue in 0.85 per cent. salt solution.	
Salt solution, 0.85 per cent. ....	5 c.c.
Sodium oxalate solution, 2 per cent. ....	2 c.c.
Add the oxalate to the salt solution, then mix with the stain and filter.	

Puncture the finger so as to get a free flow of blood, draw a good-sized drop into a red cell counting pipet, and using the stain as a diluent fill the diluting chamber. After thorough mixing, allow this to stand for ten minutes, then blow the contents of the mixing-chamber into a centrifuge tube and centrifugalize. Draw off the staining fluid with a capillary pipet, until only the sediment of cells remains. Draw the cells from the bottom of the centrifuge tube into a capillary pipet, then place a drop of these cells on the end of a clean slide which has been slightly warmed in a flame, and spread as in making ordinary blood-smears. The preparation will remain permanent indefinitely if mounted in neutral balsam or damar and not exposed to strong daylight.

In counting the cells the use of an Ehrlich eyepiece is of great assistance. A satisfactory substitute may be improvised, however, by cutting a hole about 8 mm. square in a disk of blackened cardboard and dropping this disk over the diaphragm of the ordinary eyepiece.

In the following cases of pernicious anemia, systematic determinations of the reticulated cells were made in connection with the study of the blood-picture by the usual methods, and as will be seen from the charts and the remarks in the clinical histories, an interesting relationship can be derived between the fluctuations of the red blood-cells and the curve of the reticulated cells.

The detailed histories of the cases are as follows:

#### REPORT OF CASES

**CASE 1.—History.**—M. M., a school teacher, aged 45 was admitted to the service of Dr. H. S. Patterson, Nov. 11, 1912. The chief complaints of the patient on admission were a feeling of weakness, dyspnea, palpitation of the heart and vomiting. About Jan. 1, 1912, she first noticed a feeling of languor and loss of appetite. In a short time the color of her skin and eyes alarmed her and she consulted a physician who treated her for "jaundice and weak kidneys." She was put to bed in February and remained there until April. For about a month she felt well and strong. In May she had palpitation of the heart on the least excitement and suffered from attacks of vertigo and morning headaches. Occasionally she had morning vomiting. From June to September she suffered from irregular short periods of colitis. She had been in bed for three months prior to admission, the most prominent symptoms being weakness, vomiting and anorexia.

**Examination.**—The patient is poorly nourished and acutely ill. Her complexion is pale and of a lemon-yellow tint. The sclerae have an icteroid tinge. The teeth and mouth are in good condition. The lungs are clear, the heart is of normal outline, the sounds are of good quality and are regular. There is a booming systolic murmur all over the precordium, which is loudest at the apex. There is no accentuation of the pulmonic or aortic sounds. The knee-jerks are absent. The blood-picture on admission shows red blood-cells 1,160,000; hemoglobin, 22 per cent.; white blood-cells, 5,000; polymorphonuclear leukocytes, 75 per cent.; lymphocytes, 25 per cent. The erythrocytes show marked poikilocytosis, anisocytosis, polychromatophilia and an occasional normoblast, but no megaloblasts. The reticulated erythrocytes are only 0.5 per cent. of the total, showing that no active regeneration is going on. The Wassermann reaction is negative. The analysis of the stomach contents after a test-meal shows absence of free hydrochloric acid and a very low total acidity.

For two weeks the patient continued to grow worse, and the cell-count fell rapidly.

**Treatment and Course.**—December 5 her condition was so precarious that an immediate transfusion was decided on. A count made a few hours prior to the transfusion showed that the reticulated erythrocytes had risen to 8.2 per cent., and that there was an attempt on the part of the bone-marrow to produce new cells. The majority of the reticulated cells were macrocytes. The patient received only a small amount of blood at the time of transfusion, as the accompanying counts show. After remaining for from four to five days in a semi-comatose condition, she began to improve rapidly. About a week after the transfusion sodium cacodylate grains  $\frac{3}{4}$  daily subcutaneously was started. The rapid improvement which followed the transfusion seems to imply that the stimulating influence of the presence of the donor's blood was sufficient to arouse the patient's marrow into renewed activity. It also suggests that since with the small amount

of blood given in this case a favorable result was obtained, transfusion of a large amount of blood is by no means always necessary. The patient has improved steadily up to date. Although the blood-picture has returned practically to normal, the gastric achylia is still as pronounced as at first. It was subsequently learned that about six weeks after leaving the hospital this patient underwent a relapse. She was transfused twice more, but with little benefit, and early in July she died. A very interesting point is the sharp rise in the percentage of the reticulated cells preceding the rise in the erythrocyte count and the gradual fall of the percentage of the same cells as the total erythrocyte count became higher. As seen by Chart 1, the reticulated cells gradually fall to the percentage found in normal blood as the total count approaches its maximum.

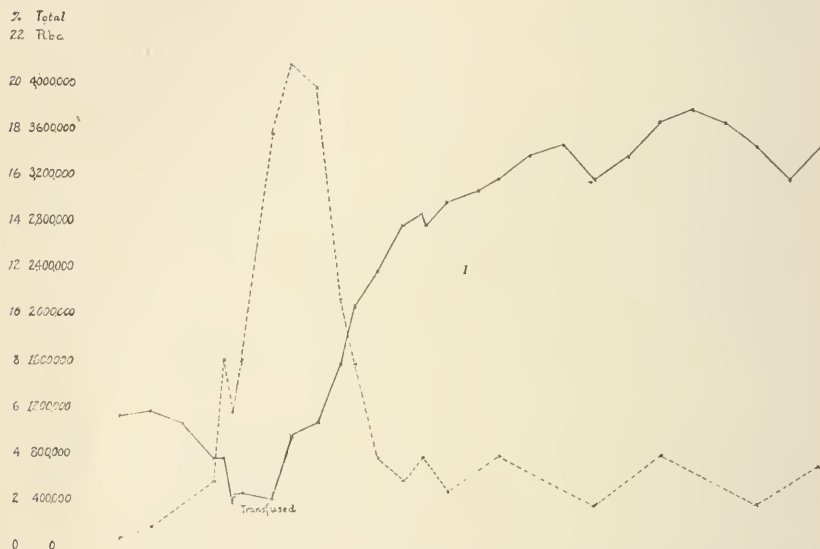


Chart 1.—Blood-findings in Case 1. In this and the following charts the solid line indicates the number of total erythrocytes, and the dotted line the percentage of reticulated cells.

CASE 2.—*History*.—M. L., a housewife aged 52, was admitted to the service of Dr. S. W. Lambert, Dec. 12, 1912. Up to the time of the onset of the present illness the patient says she has been in perfect health. In February, 1912, after a short period of failing appetite, her attention was attracted to the sallowness of the skin and the yellowish tinge of her eyes. During the weeks following a general sense of lassitude developed, and with it attacks of vomiting, at irregular intervals. During the last six months she had noticed that the vomiting is less when she is quiet in bed than when up and about her work. In April she was in bed for one month for "nervous prostration." Subsequent to the rest she was able to resume her household duties with little physical discomfort besides shortness of breath and occasionally numbness of the hands and feet. Six weeks before admission her weakness forced her to give up all work and go to bed. She has lost 37 pounds during the last year. The patient comes into the hospital "without a pain or an ache, but feeling miserably weak."

*Examination*.—The patient is pale, appears jaundiced and looks chronically ill. Her sclerae are distinctly yellow, and her left pupil is larger than the right. Her speech is slow and drawing. The teeth are in poor condition, with many missing. The lungs are clear. The heart is normal in size, but shows a faint systolic murmur, which can be heard all over the precordium, loudest at the apex

and pulmonic areas, and transmitted to the back and axilla. The analysis of the fasting stomach contents and also that of the test-meal shows achylia. The Wassermann reaction is doubtfully negative. The blood-picture is one of typical pernicious anemia. The morphology of the red cells shows poikilocytosis, anisocytosis, polychromatophilia and an occasional megaloblast and normoblast. The color index is 1.3.

*Treatment and Course.*—The patient could not be induced to take sufficient nourishment, declined steadily, and December 26 was transfused. The transfusion was very successful, both as regards the patient's immediate condition and the microscopical findings. The value of the microscope as a control in the operating-room during transfusion was demonstrated in this case. At the end of fifty minutes, when the surgeon was satisfied that the patient had received a large amount of blood, a red-cell count and hemoglobin determination showed that the amount received was almost negligible. A new anastomosis was made and in forty minutes the patient received a quantity sufficient to raise the total cell-count 1,000,000 per cubic centimeter. For a few days the patient was appreciably brighter, but the improvement was not long continued. For a month the total cell-count varied little. The vital staining of the erythrocytes showed plainly the presence of the donor's cells in the smears, for from the first

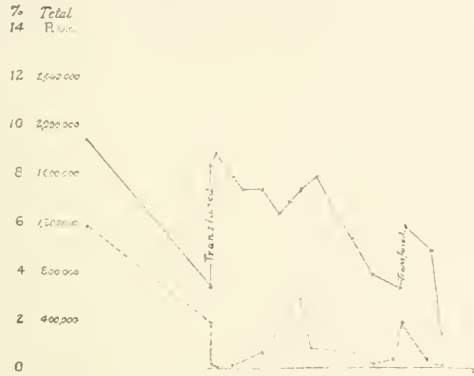


Chart 2.—Blood-findings in Case 2.

these did not take the stain as well as those of the recipient. From day to day they could be seen to have lost their hemoglobin to a greater and greater degree. After about two weeks they existed as shadow outlines of the original cells. At the end of three weeks a considerable number of these shadow cells were still present. There was no active regeneration on the part of the patient's marrow as far as could be determined by the blood-picture. The reticulated cells never reached more than 3 per cent. of the total (Chart 2) and megaloblasts and normoblasts were rarely seen. January 30 a second transfusion was done. While this time only one-half as many cells were given, the patient seemed toxic from immediately after the operation to the time of her death one week later.

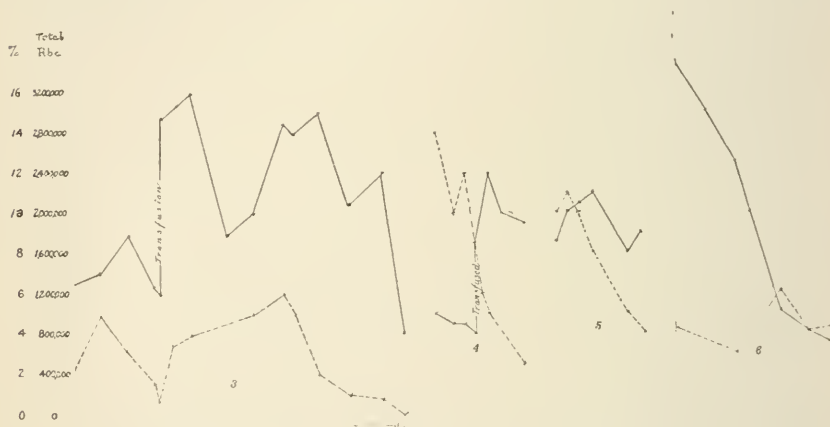
*CASE 3.—History.*—Dr. D., a Cuban physician aged 58, was admitted to the service of Dr. A. W. Hollis. In 1898 the patient was "executed" shortly before the beginning of the Spanish-American War. One bullet pierced the right shoulder and another passed through the body just above the trochanters as he fell. He was left for dead, but was later taken to the hospital in Havana, where he received treatment. Since that time he has had occasional attacks of diarrhea. During the last five years he has suffered from colitis, at irregular intervals and for periods of five to ten days, but has never regarded these as serious. Two years ago he was told that he had severe anemia. He was not incapacitated for



work, but during the last few months has lost much strength and weight. For the last two months the attacks of colitis have recurred with increasing frequency until the last two weeks, since when he has had from eight to ten stools a day, accompanied by much tenesmus. He vomits frequently and has morning headaches. His tendency to dyspnea and vertigo have necessitated his remaining in bed for the last two weeks.

*Examination.*—The patient is poorly nourished and shows signs of emaciation. The sclerae are yellow; the skin is pigmented. The mouth and teeth are in good condition. The lungs are clear. The heart is not enlarged, the sounds are weak and no murmurs are present. The abdomen shows general tenderness, especially over the region of the colon. The Wassermann reaction is negative, as is the blood-culture. The stomach contents show low free hydrochloric acid and low total acidity. The stools examined on several occasions always give positive tests for free blood. No parasites or ova are found.

*Treatment and Course.*—On admission the patient was acutely ill and much emaciated from his colitis. The colitis could not be checked after about three weeks' time; the weakness and vertigo were still prominent symptoms and transfusion was decided on.



Charts 3, 4, 5 and 6.—Blood-findings in Cases 3, 4, 5 and 6.

An artery-to-vein anastomosis was made and the blood allowed to flow for forty-five minutes. The total cell-count in the recipient was increased from 1,200,000 to 3,000,000 erythrocytes per cubic millimeter. The blood-picture before the transfusion had shown extreme poikilocytosis, anisocytosis, polychromatophilia and stippling of the erythrocytes. The immediate effect of the transfusion was apparent in the subsidence of the patient's symptoms with the exception of the colitis. This increased in severity for three or four days and then was checked. Much improvement followed for a month, the patient keeping the donor's cells and regenerating his own rapidly enough to keep the cell-count over 2,500,000. The "shadow cells" of the donor persisted for about three weeks in the blood-picture. The reticulated erythrocytes never amounted to over 6 per cent. of the total count (Chart 3). March 5 the patient on his own initiative was given salvarsan intravenously. The colitis at once again became severe, but in spite of this there was temporarily sufficient improvement to permit the patient to leave his bed. He insisted on a second dose of the salvarsan, but immediately after this the cell-count dropped and death followed in five days.

*CASE 4.—History.*—K. L., a German housewife, aged 46, admitted to the service of Dr. S. W. Lambert May 2, 1913. The patient complains of weakness and hemorrhage on entering the hospital. Her family and past histories are nega-

tive. She dates the onset of her present illness from the time about two years ago, when she suffered from "fever and chills" for several weeks. Since then she thinks that she has been losing weight and has gradually become weaker. About two months ago, at her regular menstrual period, the patient had a hemorrhage, during which she thinks she lost a large amount of blood. One month ago she discovered that she was unable to climb the stairs to her apartment because of weakness, shortness of breath and palpitation of the heart. During the last two weeks she has had three attacks of nosebleed. Six weeks ago the patient took some medicine which upset her stomach, and she has vomited almost daily since. She has numbness of the hands and feet. She is sure that she has lost 25 pounds in weight in the last year.

*Examination.*—The patient is a poorly nourished woman of middle life, who appears chronically ill. Her skin is of a deep lemon color and the mucous membranes are very pale. She is weak and prostrated. The sclerae have a slight icteroid tint. The mouth is in poor condition, containing many carious teeth. The tongue is moist and heavily coated. The lungs are clear. The heart is normal in outline. The sounds at the apex are regular and of fair muscular quality. The first sound is accompanied by a loud blowing systolic murmur, heard all over the precordium and transmitted to the axilla. The pulse suggests the Corrigan type. The patient's Wassermann is doubtfully positive and her blood-culture is negative. No gastric analysis was made because of her inability to retain food.

*Treatment and Course.*—Although there was no apparent change in the blood-picture during the first week of the patient's stay in the hospital, she grew rapidly weaker because of the inability to retain food. Over two-thirds of the nutrient enemata were expelled soon after they were given. May 10 she was transfused; the total cell-count after transfusion was 900,000 per cubic millimeter more than before. Before the transfusion the blood-picture had been one of active regeneration. The reticulated erythrocytes varied from 10 to 14 per cent. (Chart 4), and together with the presence of many normoblasts and megaloblasts showed that the bone-marrow was still actively functioning. The effects of the transfusion were transient. The following day found her in a stuporous condition, from which she did not rally. On May 19 she went into delirium, which was followed by coma and death in a few hours. As in the other cases the cells of the donor could after two or three days be distinguished from those of the recipient, and they persisted up to the time of the patient's death. In this instance the patient received what may have been an overwhelmingly large dose of blood, and it is possible that a smaller transfusion might have been more advantageous.

*CASE 5.—History.*—Mrs. L. S., a Swiss housewife, aged 42, admitted to the service of Dr. S. W. Lambert April 16, 1913, gives as her chief complaints general weakness and epistaxis. Eight years ago she was in the hospital complaining of a "goiter." After a few weeks in bed she was treated from the outpatient department of the hospital with "injections." She says that she then had "nervousness and a fluttering heart." These symptoms gradually disappeared and she was well until April, 1909. At that time she was treated in the hospital for pernicious anemia and was discharged with the diagnosis: pernicious anemia, acute thyroidism, chronic endocarditis. She was in the wards again in May, 1910, and April, 1911. Four weeks ago she was taken suddenly with a chill and fever. The doctor told her that she had "grip." When she attempted the resumption of her housework she found she did not have enough strength. Her face became puffy and her feet began to swell. She has been out of bed but one day since, and thinks that she has lost 12 pounds in the last month. The shortness of breath and palpitation of the heart have been growing worse. She has had several severe nose-bleeds in the last few days.

*Examination.*—The patient is a fairly nourished woman who appears chronically ill. The skin has an icteroid tint, and the face is slightly edematous. The sclerae are bluish-yellow. The teeth are carious and the mouth poorly kept. The lungs are clear. The outline of the heart is normal. Typical signs of mitral

BLOOD-COUNTS OF SIX PATIENTS WITH PERNICIOUS ANEMIA

Case	Date	Erythrocytes	Hemoglobin	Color Index	Leukocytes	Differential Count					Retic. Eryth. Per Cent.	Fragility		Coag. Time*
												Hemolysis		
						P.	L.	Eo.	Ba.	M.		Began Per Cent.	Complete Per Cent.	
1	11/11/12	1,160,000	22	0.95	5,000	75	25	..	..	..	0.5	0.40	0.34	2' 20"
	11/18/12	1,200,000	20	0.83	4,600	83	16	1	..	..	1.0	....	....	2' 15"
	11/25/12	1,100,000	20	0.90	4,000	78	20	1	1	..	...	....	....	....
	12/ 2/12	800,000	20	1.2	3,000	80	19	1	..	..	3.0	....	....	....
	12/ 4/12	800,000	20	1.2	....	..	..	..	..	..	8.2	....	....	....
	12/ 5/12†	400,000	12	1.5	....	..	..	..	..	..	6.0	....	....	....
		500,000	12	1.2	....	..	..	..	..	..	...	....	....	....
	12/ 6/12	500,000	15	1.5	3,000	78	20	1	1	..	7.0	....	....	....
	12/13/12	480,000	15	1.5	6,000	60	40	..	..	..	8.2	....	....	....
	12/17/12	1,000,000	20	1.0	3,000	65	33	1	..	1	8.0	....	....	....
	12/23/12	1,100,000	35	1.6	5,000	82	18	..	..	..	18.0	....	....	....
	12/28/12	1,600,000	45	1.4	....	..	..	..	..	..	21.0	....	....	....
	12/31/12	2,100,000	50	1.2	6,000	70	29	..	1	..	20.0	....	....	....
	1/ 6/13	2,400,000	50	1.0	....	..	..	..	..	..	11.0	....	....	....
	1/11/13	2,800,000	50	0.9	6,200	68	31	1	..	..	8.0	....	....	....
	1/15/13	2,900,000	65	1.1	....	..	..	..	..	..	4.0	....	....	....
	1/16/13	2,800,000	65	1.1	....	..	..	..	..	..	3.0	....	....	....
	1/20/13	3,000,000	65	1.0	....	..	..	..	..	..	4.0	....	....	....
	1/27/13	3,100,000	67	1.0	6,000	74	25	1	..	..	2.5	....	....	....
	2/ 3/13	3,200,000	67	1.0	....	..	..	..	..	..	3.0	....	....	....
	2/10/13	3,400,000	70	1.0	7,200	70	28	2	..	..	...	....	....	....
	2/17/13	3,500,000	70	1.0	....	..	..	..	..	..	1.7	....	....	....
	2/24/13	3,200,000	72	1.1	6,800	57	43	..	..	..	...	....	....	....
	3/ 3/13	3,400,000	75	1.1	4,600	62	37	1	..	..	4.0	....	....	....
	3/10/13	3,700,000	77	1.0	....	..	..	..	..	..	...	....	....	....
	3/17/13	3,800,000	77	1.0	....	..	..	..	..	..	...	....	....	....
	3/24/13	3,700,000	80	1.0	5,000	67	31	1	1	..	1.8	....	....	....
	3/31/13	3,500,000	77	1.1	....	..	..	..	..	..	...	....	....	....
	4/ 6/13	3,200,000	75	1.1	....	..	..	..	..	..	3.5	....	....	....
	4/13/13	3,500,000	..	...	....	..	..	..	..	..	2.1	....	....	....
2	12/14/12	1,900,000	50	1.3	4,800	75	23	1	1	..	6.0	0.42	0.38	2' 26"
	12/26/12†	700,000	25	1.6	3,600	68	32	..	..	..	2.0	....	....	....
		1,700,000	45	1.2	....	..	..	..	..	..	0.8	....	....	....
	12/27/12	1,800,000	40	1.1	2,200	58	40	1	..	1	0.25	....	....	1' 57"
	12/30/12	1,600,000	32	1.0	....	..	..	..	..	..	0.28	....	....	....
	1/ 1/13	1,500,000	30	1.0	3,000	..	..	..	..	..	...	....	....	....
	1/ 3/13	1,500,000	35	1.1	3,500	76	23	1	..	..	0.76	....	....	....
	1/ 6/13	1,300,000	35	1.3	....	..	..	..	..	..	...	....	....	....
	1/ 8/13	1,400,000	35	1.2	....	..	..	..	..	..	3.0	....	....	....
	1/10/13	1,500,000	35	1.1	....	..	..	..	..	..	1.0	....	....	....
	1/13/13	1,600,000	32	1.0	3,600	72	28	..	..	..	0.8	....	....	....
	1/20/13	1,100,000	22	1.0	....	..	..	..	..	..	0.37	....	....	....
	1/24/13	800,000	20	1.2	2,300	33	64	2	1	..	0.25	....	....	....
	1/30/13†	700,000	18	1.3	....	..	..	..	..	..	0.45	....	....	....
		1,200,000	20	0.83	....	..	..	..	..	..	2.0	....	....	....
	2/ 5/13	1,000,000	20	1.0	3,600	42	57	1	..	..	0.5	....	....	....
	2/ 7/13	320,000	15	2.3	1,100	31	67	..	1	1	0.25	....	....	....

\* By the Dale and Laidlaw method. (Jour. Path. and Bacteriol., 1912, xvi, 351.)

† Transfusion.      ‡ Salvarsan.

## BLOOD-COUNTS OF SIX PATIENTS WITH PERNICIOUS ANEMIA.—(Continued)

Case	Date	Erythrocytes	Hemoglobin	Color Index	Leukocytes	Differential Count					Retic. Eryth. Per Cent.	Fragility		Coag. Time*
												Hemolysis		
						P.	L.	Eo.	Ba.	M.		Began Per Cent.	Complete Per Cent.	
3	1/14/13	1,380,000	35	1.3	3,300	58	42	..	..	..	2.3	0.40	0.28	2' 20"
	1/20/13	1,480,000	35	1.2	6,300	69	31	..	..	..	5.0	....	....	....
	1/27/13	1,800,000	40	1.1	4,900	59	40	1	..	..	3.3	....	....	....
	2/ 3/13	1,280,000	40	1.6	3,600	46	52	1	1	..	1.6	....	....	....
	2/ 4/13†	1,200,000	35	1.4	.....	..	..	..	..	..	0.9	....	....	....
		3,000,000	60	1.0	3,800	48	52	..	..	..	1.0	....	....	....
	2/ 7/13	3,100,000	60	0.96	3,600	52	46	1	..	1	3.5	....	....	....
	2/10/13	3,200,000	65	1.0	....	..	..	..	..	..	4.0	....	....	....
	2/18/13	1,600,000	48	1.5	6,000	64	33	3	..	..	...	....	....	....
	2/25/13	2,000,000	60	1.5	.....	..	..	..	..	..	5.0	....	....	....
	3/ 3/13	2,900,000	70	1.2	4,500	62	37	1	..	..	6.0	....	....	....
	3/ 5/13‡	2,800,000	70	1.2	....	..	..	..	..	..	5.0	....	....	....
	3/11/13	3,000,000	75	1.2	....	..	..	..	..	..	2.0	....	....	....
	3/19/13	2,120,000	55	1.3	4,600	68	32	..	..	..	1.0	....	....	....
3/24/13‡	2,400,000	60	1.2	.....	..	..	..	..	..	0.76	....	....	3' 15"	
3/29/13	890,000	25	1.3	1,300	28	68	4	..	..	?	....	....	....	
4	4/ 2/13	1,000,000	20	0.9	10,000	48	50	2	..	..	14.0	0.40	0.30	2' 59"
	4/ 6/13	900,000	25	1.2	5,000	50	47	3	..	..	10.0	....	....	....
	4/ 8/13	900,000	25	1.2	9,000	65	35	..	..	..	12.0	....	....	....
	4/10/13†	800,000	25	1.9	....	..	..	..	..	..	..	....	....	....
		1,700,000	35	0.9	4,800	62	34	3	1	..	6.0	....	....	....
	4/13/13	2,400,000	50	0.9	4,500	58	41	..	..	..	5.0	....	....	....
	4/15/13	2,000,000	40	0.9	....	..	..	..	..	..	..	....	....	....
	4/19/13	1,900,000	45	1.0	3,200	46	52	1	1	..	2.1	....	....	....
5	4/17/13	1,760,000	40	1.1	3,000	52	47	1	..	..	10.0	0.38	0.32	2' 10"
	4/19/13	2,050,000	55	1.3	4,600	60	38	1	1	..	11.0	....	....	....
	4/21/13	2,100,000	55	1.3	4,200	64	34	2	..	..	10.0	....	....	....
	4/24/13	2,200,000	55	1.2	4,500	68	32	..	..	..	8.0	....	....	....
	5/ 2/13	1,600,000	45	1.4	.....	..	..	..	..	..	5.0	....	....	....
	5/ 5/13	1,800,000	50	1.3	4,800	70	27	1	2	..	4.0	....	....	....
6	4/30/12	4,000,000	75	0.90	5,000	74	26	..	..	..	...	0.38	0.28	....
	5/17/12	3,600,000	60	0.83	4,500	60	38	1	1	..	...	....	....	....
	6/14/12	3,680,000	65	0.88	6,640	64	36	..	..	..	...	....	....	....
	7/18/12	3,700,000	72	0.97	2,100	70	30	..	..	..	...	....	....	....
	9/16/12	3,800,000	75	0.98	3,400	60	39	1	..	..	...	....	....	....
	10/ 3/12	3,500,000	65	0.92	4,200	58	41	1	..	..	...	....	....	....
	11/29/12	3,500,000	55	0.80	5,500	60	40	..	..	..	4.2	....	....	2' 24"
	12/ 6/12	3,000,000	69	1.1	8,000	64	36	..	..	..	...	....	....	....
	12/13/12	2,500,000	75	1.5	5,800	66	32	..	2	..	3.0	....	....	....
	1/24/13	1,000,000	20	1.0	1,400	49	51	..	..	..	6.0	....	....	....
	1/30/13	800,000	15	1.0	3,500	51	48	1	..	..	4.0	....	....	....
	2/ 2/13	750,000	20	1.3	2,100	37	62	1	..	..	4.2	....	....	2' 37"



stenosis and regurgitation are present. The right kidney is palpable on deep inspiration. Examination of a test-meal shows the absence of free hydrochloric acid, and a total acidity of 10; no blood or lactic acid. The Wassermann reaction is negative, as is the blood-culture.

This patient remained in the hospital for a little over two weeks. She was apparently comfortable and wanted to return to her own home and resume her housework. Although the reticulated erythrocytes were present in such large percentages and indicated an active marrow, there were surprisingly few nucleated red cells. The erythrocytic picture was typically that of pernicious anemia (Chart 5). The constancy of the red blood-cell count showed that the regeneration was taking care of the cell destruction. The resistance of the red blood-cells to hypotonic salt solutions was increased, for hemolysis did not begin until a dilution of 0.38 per cent. was reached, and to cause complete hemolysis a solution of 0.32 per cent. was required.

**CASE 6.—History.**—Miss C., a nursemaid, aged 43. was admitted to the service of Dr. S. W. Lambert. Beginning in September, 1911, the patient had a severe attack of diarrhea, which lasted six weeks. She had as many as fifteen movements a day. The stools contained both blood and mucus. Immediately following this she had palpitation of the heart on exertion, and was very nervous and easily excited. She was admitted to the hospital April 29, 1912, with a swelling on the front of the neck. She complained of loss of weight in spite of her good appetite. She also had marked polyuria. The superior thyroid arteries were ligatured, and while it was thought that there was improvement, the edema of the feet, nervousness, pain in the back and the weakness did not disappear. She remained in the medical wards from Sept. 16, 1912, to Dec. 21, 1912, having been transferred there from the surgical division. She was discharged on December 21 as improved. On Jan. 24, 1913, she was again admitted to the medical service, complaining of a "noise in the ears, nausea and weakness." Dyspnea was present on exertion, but no cough. For two weeks prior to admission she had been annoyed by a buzzing in the head that seemed to start in the ear and radiate toward the neck. She was very nervous, but had no sweating. The increasing weakness was becoming more and more noticeable.

**Examination.**—The patient is poorly nourished, acutely ill and looks "nervous." The skin is jaundiced. The eyes show slight exophthalmos, the sclerae have an icteroid tint, but the other eye-signs of Graves' disease are absent. There is a fulness in the thyroid region on palpation. A blowing systolic murmur is present in the gland. The lungs are clear. The heart is normal in outline, regular, forcible and rapid. All the signs of mitral regurgitation are present. Over the precordial area, and especially in the second left interspace, a to-and-fro friction rub is heard. There is edema of the lower extremities, especially on standing. In the right retina there were three fresh hemorrhages. The Wassermann reaction and two blood-cultures are negative.

**Course.**—The patient had constant fever, between 99 and 102 F. At first the diagnosis was somewhat in doubt; but a careful study of the blood showed that the anemia was of the pernicious type. The reticulated erythrocytes were never higher than 6 per cent. (Chart 6), and with the extreme degree of anemia the prognosis was considered bad. In this case also the resistance of the red blood-cells was increased and the coagulation time was much prolonged. The patient grew gradually weaker and died before a donor for transfusion could be obtained.

A summary of the findings in the six cases is given in the accompanying table.

#### GENERAL CONSIDERATIONS

It is unfortunately still true that any discussion of the treatment of pernicious anemia must be prefaced by the statement that the disease is

an incurable one, and that the instances in which complete recovery is alleged as having followed some particular method of treatment, are to be regarded as explicable either on the ground of faulty diagnosis or insufficient observation. Nor is this altogether astonishing in view of the vagueness of our notions concerning the real nature of the malady. In dealing with other, secondary, forms of anemia, the conditions are different, for we are able to train our therapeutic efforts in two directions, first, toward removing the underlying cause of the trouble, and, second, toward influencing the regenerative functions of the blood-forming tissues. In the case of pernicious anemia, however, our ignorance of its etiology converts all our attempts at causal therapy into the purest empiricism (leaving out of account the mooted cases of bothrioccephalus, syphilitic and puerperal pernicious anemia), and impels us to the conclusion that for the present we shall accomplish most by endeavoring to stimulate the flagging hematopoietic energies of the bone-marrow.

Of the many measures having this object in view, transfusion of blood in one form or another seems to offer particularly promising possibilities, and the highly encouraging results reported by many clinicians (as for example, Bovaird,<sup>3</sup> Hürter,<sup>4</sup> Hansen,<sup>5</sup> Weber,<sup>6</sup> Morawitz,<sup>7</sup> Sachs,<sup>8</sup> Gulland and Goodall,<sup>9</sup> etc.) more than justify a wider application of the procedure.

Hürter believes in transfusing early and always recommends the operation just as soon as the blood-picture ceases to show evidence of active regeneration. Undoubtedly, some portions of the bone-marrow retain their functional capacity longer than others, but if the delay is too great these also lapse into a state of inertia from which they cannot be aroused, even by the penetrating stimulus of the hemopoietins in the transferred blood.

The history of transfusion is a long one, and those who are interested in its details will find them set forth in an excellent article on the subject by Werner Schultz,<sup>10</sup> which gives also a complete bibliography up to 1910. That it did not require the era of vascular surgery to popularize the measure, is shown by the fact that in 1875, Landois<sup>11</sup> was able to collect from the literature records of 347 transfusions from one human being to another. At about this period the same great physiologist, together

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3. Bovaird: *Med. Rec.*, 1911, lxxxi, 239.

4. Hürter: *Med. Klin.*, 1911, Beiheft, No. 12.

5. Hansen: *Verhandl. d. deutsch. Kong. f. inn. Med.*, 1911, p. 141.

6. Weber: *Deutsch. Arch. f. klin. Med.*, 1909, xcvii, 165.

7. Morawitz: *München. med. Wchenschr.*, 1907, liv, 767.

8. Sachs: *Ztschr. f. Geburtsh. u. Gynäk.*, 1909, lxiv, 336.

9. Gulland and Goodall: *The Blood*, New York, E. B. Treat & Co., 1912, p. 133.

10. Schultz: In Grawitz: *Klinische Pathologie des Blutes*, Leipsic, 1911, p. 381.

11. Landois: *Transfusion des Blutes*, Leipsic, 1875.

with Ponfick,<sup>12</sup> Panum<sup>13</sup> and others, established the fact that transfusion with animal blood which previously had been widely resorted to, was dangerous, and that clinically only human blood could safely be used. Later the question arose as to whether whole blood or defibrinated blood should be employed, and this has been made the subject of extensive discussion and difference of opinion. Many of the German authors still prefer to inject defibrinated blood, regarding the greater ease of working with it as offsetting the danger of introducing the deleterious substances shown to be present by Köhler,<sup>14</sup> Edelberg,<sup>15</sup> Schultz<sup>16</sup> and others. Later work by Morawitz,<sup>17</sup> Fuld<sup>18</sup> and Moldovan<sup>19</sup> has proved that these hazards may largely be avoided by allowing the defibrinated blood to stand from one-half to one hour before injecting, for under these conditions the injurious bodies produced during the act of defibrination become altered into harmless modifications. Morawitz<sup>7</sup> and Schultz<sup>10, 16, 20</sup> were also able to show that the greatest danger to be provided against was the presence of untoward bodies of the nature of iso-agglutinins or iso-hemolysins in the two bloods, and that, therefore, no transfusion should be done unless the corpuscles and serum of the donor and recipient had first been tested reciprocally. In this connection it is worth noting that Hopkins<sup>21</sup> has reported a case in which, although the cells of the donor were only slightly agglutinated by the recipient's serum, smears made immediately after the transfusion showed extensive destruction of the donor's cells by phagocytes from the recipient. Curtis and David<sup>22</sup> after experimental work on dogs do not consider defibrinated blood suitable for human transfusion, and Pike, Guthrie, and Stewart<sup>23</sup> have found that on standing a number of hours, defibrinated blood undergoes alterations affecting the corpuscles and impairing its nutritive and bactericidal qualities.

Further evidence of the changes occurring in defibrinated or clotted blood is offered by the work of Stevens and Lee,<sup>24</sup> O'Connor,<sup>25</sup> Brodie,<sup>26</sup> Sollmann,<sup>27</sup> and Stewart and Harvey<sup>28</sup> on the differences in the pressor

12. Ponfick: Virchows Arch. f. path. Anat., 1875, lxii, 273.
13. Panum: Virchows Arch. f. path. Anat., 1863, xxvii, 240.
14. Köhler: Inaug. Diss., Dorpat, 1877.
15. Edelberg: Arch. f. exper. Path., 1880, xii, 283.
16. Schultz: Deutsch. Arch. f. klin. Med., 1905, lxxxiv, 541.
17. Morawitz: Ergebn. d. Physiol., 1905, iv, 307.
18. Fuld: Zentralbl. f. Physiol., 1903, xvii, 529.
19. Moldovan: Deutsch. med. Wehnschr., 1910, xxxvi, 2422.
20. Schultz: Berl. klin. Wehnschr., 1910, xlii, 1407.
21. Hopkins: THE ARCHIVES INT. MED., 1910, vi, 270.
22. Curtis and David: Surg., Gynec. and Obstet., 1912, xv, 476.
23. Pike, Guthrie and Stewart: Jour. Exper. Med., 1908, x, 371.
24. Stevens and Lee: Bull. Biol. Lab., Johns Hopkins Univ., 1884.
25. O'Connor: Arch. f. exper. Path., 1912, lxvii, 195.
26. Brodie: Jour. Physiol., 1900, xxvi, 48.
27. Sollmann: Am. Jour. Physiol., 1905, xiii, 291.
28. Stewart and Harvey: Jour. Exper. Med., 1912, xvi, 103.

substances in blood before and after defibrination or clotting. Clinical observation confirms this and various authors, such as Hürter, state that the therapeutic effects of whole blood are distinctly better than those following the use of defibrinated blood.

While the development of the art of vascular surgery to its present high degree, including as it does the possibility of transferring absolutely fresh and, as far as we know, unaltered blood from one individual to another, would appear to make all further consideration of the use of defibrinated blood unnecessary, it must be admitted that from the standpoint of the clinician, these surgical transfusions are still far from ideal. In spite of the various highly ingenious devices that have been suggested for facilitating the operation, it is usually time-consuming, except in the hands of particularly skilful operators, and is a considerable ordeal to both donor and recipient. This fact, together with the sacrifice of a vessel which is entailed, makes donors difficult to find and causes them to hesitate about serving a second time; but the most serious drawback lies in the difficulty of estimating the amount of blood transferred. Counting the recipient's cells and determining the amount of hemoglobin at regular intervals, during the transfusion, should never be omitted, but this is at best only a halting method of gauging the blood-flow.

Both our own observations and those of others lead to the conclusion that more is to be expected from small transfusions repeated at rather frequent intervals, than from a single one of large amount (Weber,<sup>6</sup> Henrot,<sup>29</sup> Hürter<sup>4</sup>). There is even a likelihood that, as in the case of other stimulants, unnecessarily large doses of blood may actually be injurious, and as it were, overwhelm the last flicker of regenerative activity still persisting in the marrow. Or, there is also the possibility that the abrupt change from a state of extreme blood-poverty to one in which there is a great profusion of vigorously functioning cells, by relieving the bone-marrow from the insistent demands to which it has long become habituated, allows it to subside into a condition of hopeless inactivity.

Several possibilities suggest themselves in explanation of the effect of the transfusion. Either the transferred blood is functionally active directly as a transplanted tissue, and its value, therefore, is proportionate to the length of time its elements remain intact, or it is conceivable that it may have an antitoxic action on the etiological factor of the disease. Turk,<sup>30</sup> for example, believes that the new blood, being less resistant than the patient's cells, combines with some putative hemolytic principle present, and so by temporarily protecting the bone-marrow, allows it

29. Henrot: *Bull. de l'Acad. d. méd., Paris*, 1913, lxxvi, 38.

30. Türk: *Vorlesungen über klinische Hämatologie*, Vienna, 1912. ii, part 2, p. 701.



opportunity to restore some of the lost cells. In the opinion of most authors, however, the transferred blood acts as a stimulant, especially to the blood-forming organs, and at least for a time, revives the power of blood regeneration.

Carnot<sup>31</sup> attempted to increase the effect of these stimulating bodies or hemopoietins by using blood from animals that shortly before had been subjected to a venesection, but while there seems to be some experimental basis for believing that this is possible, further work by Gibelli<sup>32</sup> and by Larrabee<sup>33</sup> has shown that the procedure offers little hope of being valuable clinically. The same thing is true of the efforts of Courmont and André,<sup>34</sup> and other French workers, to obtain increased stimulation of hematopoiesis by administering small doses of hemolytic serums. In this connection it is of interest to note that Walter<sup>35</sup> has reported a case of pernicious anemia treated effectively by repeated injections of blood taken from a patient with polycythemia.

Although, of course, it is perfectly possible and even probable that the benefit following transfusion is more or less a composite of all these factors, it seems most likely—and this opinion is shared by most writers on the subject—that it is the stimulation of the bone-marrow that is chiefly effective.

While the transferred cells may serve to tide over a more or less acute emergency, their life in the new host is certainly too short to account for the steady rise in red cells that is frequently observed during a considerable period following the transfusion, and it is hardly conceivable that this is merely the result of improved nutritional conditions. The persistence of the new cells has been variously estimated by different writers, but is generally supposed to be about three or four weeks. Our own observations, by means of the method of vital staining, incline us to the view that in most instances they do not last so long, two or three weeks being the usual period in our cases, and during the latter part of this time they are so poor in hemoglobin as to be of comparatively little functional value.

Although some clinicians (Esch,<sup>36</sup> Huber,<sup>37</sup> Mann<sup>38</sup>) have had good results from the subcutaneous or intramuscular injection of defibrinated blood, this has not been our experience, or that of the majority of writers. Both from our own observations and from a survey of the literature, the

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31. Carnot: *Folia haematol.*, 1909, vii, 75.

32. Gibelli: *Arch. f. exper. Path. u. Pharmacol.*, 1911, lxxv, 284.

33. Larrabee: *Jour. Med. Res.*, 1911, xxiv, 345.

34. Courmont and André: *Folia haematol.*, 1904, i, 389.

35. Walter: *Med. Klin.*, 1911, vii, 728.

36. Esch: *Deutsch. med. Wehnschr.*, 1911, xxxvii, 1943.

37. Huber: *Deutsch. med. Wehnschr.*, 1910, xxxvi, 1077, and *Verhandl. Deutsch. kong. f. inn. Med.*, 1910, xxvii, 147.

38. Mann: *Wien. med. Wehnschr.*, 1911, lxi, 580.

conclusion seems justified that the optimum effect is to be expected from the repeated intravenous administration of rather small measured amounts of undefibrinated blood. The desideratum appears to be the employment of a method by means of which this can be effected with less difficulty for all concerned than is the case when a vascular anastomosis is undertaken, so that the measure may be made more easily applicable to the conditions of ordinary practice. Over twenty years ago von Ziemssen<sup>39</sup> reported numerous cases in which transfusion was successfully employed by such a method. It consisted in having one operator thrust a needle through the skin into the vein of the recipient, while another operator performed the same action on the donor. Three glass syringes of 25 c.c. capacity were kept constantly in action, one being filled with blood from the donor, the other being emptied into the vein of the recipient, while the third was being washed out with sterile salt solution by a third person before being again applied to the needle in the donor's vein. By this procedure von Ziemssen was able to transfer any desired amount of unaltered blood and to measure it accurately. Moritz,<sup>40</sup> in 1911, emphasized the advantages of the method and introduced some improvements in the technic which facilitated the puncture of the vein. Still more recently, Lindeman,<sup>41</sup> using the same principle, has devised a set of cannulas by the aid of which even small veins may easily be entered, and the danger of wounding the intima is reduced, and has reported thirty-six transfusions done without a mishap, one of them in a child of seven weeks.

#### CONCLUSIONS

1. The transfusion of physiologically unaltered blood is one of the most promising forms of palliative treatment available in pernicious anemia. The number of cases on record in which a remission of notable degree and considerable duration has followed immediately on a transfusion is so great as to make it impossible to regard these results merely as coincidences. If proper precautions are taken to select a healthy donor and by the usual tests for isohemolysins and isoagglutinins the serum and corpuscles of donor and recipient are found mutually congenial, there is no danger, and the measure should be employed earlier in the disease instead of waiting until the patient is in a desperate condition.

2. There is evidence in favor of the view that greater judgment and accuracy are needed in determining the amount of blood to be trans-

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39. Von Ziemssen: *Deutsch. Arch. f. klin. med.*, 1902, l. 491. *Verhandl. d. Deutsch. kong. f. inn. Med.*, 1892; *München. med. Wehnschr.*, 1894, xli. 349; 1895, xlii, 301.

40. Moritz: *München. med. Wehnschr.*, 1911, lviii. 393.

41. Lindeman: *Am. Jour. Dis. Child.*, 1913, vi, 28.

ferred. It is quite possible that too large an amount of transferred blood may be injurious, and that more benefit is to be expected from small doses introduced at intervals to be determined by the progress of the patient.

3. The enumeration of the reticulated cells by means of the method of vital staining affords a useful means of gauging the hemopoietic activity of the bone-marrow, and by watching the patient's progress in this way the indications for and effects of, various therapeutic measures can be well determined and supervised.

## THE PATHOGENESIS OF THE CONTRACTED KIDNEY \*

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In order to obtain a comprehensive insight into the pathogenesis of the various types of contracted kidney in man, it is first of all necessary to attack the problem from the experimental standpoint, and to attempt to produce in animals artificially the identical types of contracted kidney which are observed in human pathology. Unfortunately, up to the present time this has been possible only to an exceedingly limited extent. But in this regard certain pieces of work of the past year or two have served to advance our knowledge, amongst which that of my friend Ophüls in San Francisco, of my colleague Mackenzie in Toronto, of their pupil Dickson, and of Christian are especially worthy of mention.

On the other hand, more exact studies concerning the finer morphological structure of the kidney have appeared almost simultaneously in France and in Germany, and these undoubtedly must now form the essential basis for all experimental work. I have also occupied myself with similar studies for several years past, the results of which have in large part been published during the previous year by my pupil Suzuki. My lecture therefore logically resolves itself into three parts:

1. What is our knowledge concerning the morphological structure and the function of the normal kidney?
2. What particular structural elements of the kidney must be damaged to produce chronic contracted kidneys in animals?
3. What is our knowledge concerning the chief types of human contracted kidney?

I shall begin first of all with the question concerning the morphological structure of the renal tubules. As regards the construction of the renal tubular system, we have been accustomed to differentiate the following structural groups: The capsular epithelium, the convoluted tubules of the 1st order (the "*Hauptstücke*"), the Henle's loop, the convoluted tubules of the 2d order (the "*Schaltstücke*") and the collecting tubules.

But if we are to take into consideration the newer histological studies and those founded on modern reconstruction technic—and I refer chiefly to those of Policard and Peter—we must elaborate the

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\* The Cartwright Lectures for the year 1913, of the Association of the Alumni of the College of Physicians and Surgeons, Columbia University.

\* Submitted for publication July 21, 1913.



above classification still further. For Peter divides the ascending limb of Henle's loop into a lower cloudy and an upper transparent portion. He also calls that narrow tubule which unites the ascending limb of Henle's loop with the *Schaltstück*, and which usually lies adherent to the glomerular capsule at its hilus, the *Zwischenstück* or connecting piece. And, finally, he differentiates the primary collecting tubules from the main collecting tubules, as have some previous authors.

In contrast to this complicated composition of the more distal portions of the tubular system, the *Hauptstück* (tubulus contortus of the first order) has generally been considered in the literature as an absolute unit; or, at the very most, only the terminal end (the spiral piece of Schweigger-Seidel, *partie terminale* of Policard) was distinguished from the rest of the *Hauptstück*. Even Peter in his schemata of the renal structure in the various animals treats the *Hauptstück* as a single unit, only giving the terminal portion a special distinction in the cat. Indeed, we find exceedingly scanty information in the literature concerning the finer morphological differences which distinguish this terminal portion.

On the basis of the results obtained from a study of Altmann preparations and vital staining with carmin made in conjunction with Dr. Suzuki, I am now prepared to advise a still further division. For it could be easily determined that in all the animals studied (the rabbit, guinea-pig, rat, mouse, pigeon, to a certain extent also the cat and the hedgehog), the *Hauptstück* (convoluted tubule of the first order) can be divided into at least three parts and possibly four.

The first, or proximal portion, when stained by Altmann's method, is characterized by an extraordinarily dense and deep staining rod-like structure. On the other hand, the second or middle portion is characterized by a much more diffuse and more delicate rod-like structure, whereas in the third or distal portion a gradually more and more marked solution into granules takes place, which become progressively more diffuse in distribution. This differentiation into three (or perhaps four) parts stands out still more distinctly in the carmin preparations, so that at first glance one can immediately tell which tubules in the cortex belong to the portions immediately efferent from the glomerulus and which represent the transition to the descending loop. In the rabbit one can still further divide the third or distal portion into a spiral and a straight part.

On the basis of these specific stains, I can only confirm the observation of Peter concerning the more compact arrangement of the tubular convolutions in the deeper glomerular levels, as compared with those lying nearer the surface of the organ. To my mind, the recognition of the characteristic structure of the terminal portion of the *Hauptstück* is

particularly important, for, as Peter has also pointed out, the fact that it runs deep into the medulla enables one to confuse it easily with the ascending limb of Henle's loop — a confusion which in certain recent works on vital staining has actually occurred.

Because of the fact that up to the present time the vital staining has been especially used in studying the secretory processes in the kidney, it seemed essential to know exactly where and in what manner the vital staining dyestuffs are excreted. From amongst the many dyestuffs that have previously been used, we have purposely chosen carmin, though for the sake of comparison we have also made use of trypanblue. It is, of course, impossible for me to give you the details of each of the exceedingly numerous varieties of experiments performed, nor is it possible at present to describe to you the historical development of this subject or the differences of opinion which still exist on important questions. I desire merely to mention that it was the work of Ribbert and of Arnold which above all others formed for us the starting point for further investigations.

One fact which I desire to emphasize most strongly is that the majority of the authors, though by no means all, have failed to make a sufficiently sharp distinction between the actual excretion of the dyestuff and the staining of the granules. This distinction is probably one of the most important contributions of our experiments. If the staining of the granules is taken as the index of the dyestuff excretion, then the excretion of carmin may be expected to reach its height twelve to twenty-four hours after the administration of the usual dose employed, for that is the time when a distinct staining of the granules in the cells is first to be observed. On the other hand, since it can be easily determined that only the epithelium of the *Hauptstücke* shows a granule staining, it seems self-evident that the excretion of the dyestuff takes place through just these cells and through no others. Incidentally, it must here be mentioned, that all observations which would seem to indicate that an excretion of carmin also occurs in the ascending limb of Henle's loop or in the *Schaltstücke* are based on an error. We should also prefer to believe that similar observations in regard to other dyestuffs such as toluidinblue, trypanblue, etc., are also due to false interpretation of the findings. What has been taken to be the ascending loop is nothing else than the distal portion of the *Hauptstück*. And in the *Schaltstück* or in yet more distant divisions of the tubular labyrinth, the dyestuff occurs intracellularly only, when for other reasons we have a right to assume that resorption has taken place.

Indeed, on the results of constantly repeated comparisons of Altmann preparations and carmin preparations from the same kidney, we assert that secretory granule staining occurs only in the epithelium of the *Hauptstück* and in the very beginning of the descending loop. The very

fact that the *Hauptstück* stands forth so conspicuously in the vitally stained preparations naturally indicates the possession of a special functional capacity for the excretion of dyestuffs. The variety of the observations in regard to the histological pictures observed during the process of carmin excretion is, in our opinion, to be explained by differences in the doses employed or in the duration of the experiment. If the carmin excretion be systematically followed at intervals of ten minutes, thirty minutes, one hour, etc., for several days or weeks, the following result is obtained. The intensity of the color of the urine and the repeated finding of numerous deeply stained casts of carmin in the collecting tubules of the kidney under observation, definitely indicate that the greater part of the dyestuff is excreted within the first half hour, or at most within the first hour after the injection. In microscopical preparations of kidney obtained after this time, the casts of carmin are either very scarce or entirely absent. Yet in this period when the chief secretion is taking place, no staining of the specific granules is to be seen, or at best only an extremely faint staining. Occasionally, when the dose administered is not too large, no morphological changes whatever are to be seen in the cells, which would indicate a carmin excretion. But if the carmin excretion is very marked, the picture which has been repeatedly described by A. Schmidt, Ribbert and others is to be seen; namely, pink staining of the brushlike marginal membrane (the *Bürstensaum*), together with an irregular granular precipitate at the base and on the surface of the brushlike margin itself.

The Altmann preparations show that in this period the specific rod-like or granular structure of the cells is unaltered. They demonstrate conclusively that the irregular granular deposit of carmin just described has absolutely nothing to do with the real cellular granules, but represents merely an increased concentration and precipitation within the brushlike marginal membrane which limits the cell interior from the lumen.

The carmin casts which are to be seen in the collecting tubules and occasionally also in the Henle loops present an entirely different appearance, consist of very much finer or coarser granules, and are always more or less uniform in size. The above mentioned facts all indicate that the dyestuff passes through the epithelial cells of the *Hauptstücke* in solution, and that only in the lumen of the more distal portions of the tubular system is it thrown down in granular form, due on the one hand to the slowing of the urinary current and on the other to resorptive processes. Here it is usually found embedded in albuminous material. Numerous authors, and more particularly Ribbert, have shown that a resorptive phenomenon probably takes place in the Henle loops, the *Schaltstücke* and to some extent also in the collecting tubules. But the final proof that it actually does take place is to be found in the fact that if the

experiment has lasted some length of time the epithelium of these tubules usually shows the presence of carmin granules, whereas in their lumina casts are still present. The irregularity of their distribution and their coarse appearance render it an easy matter to distinguish them from the secretory granule staining in the epithelium of the *Hauptstück*.

Although it now seems probable that during the main secretory period the excretion of carmin occurs in soluble form, the question must still be answered whether this excretion occurs exclusively through the epithelium of the *Hauptstück*, or whether it can also take place elsewhere as well, as, for example, through the glomeruli. As is well known, the occurrence of such a secretion through the glomeruli is at the present time denied. But in the mouse, if the carmin administration be pushed, carmin precipitate can even be encountered in the capsular space. Further, although all the epithelium of the *Hauptstücke* be severely damaged, as, for example, by chromium, a relatively rapid excretion of carmin will nevertheless still take place. The two facts speak for the assumption that the carmin is excreted in soluble form, not only through the epithelium of the *Hauptstück*, but also through the glomeruli, although probably in greater dilution. The fact that this excretion is ordinarily invisible does not speak against it, for if smaller doses are used the excretion can also take place through the epithelium without leaving behind any morphologically demonstrable traces.

We thereby assume a position to a certain extent opposing the present-day teaching, more especially as we desire to emphasize that the period of secretory activity must be sharply distinguished from the period of storing up—the “*Speicherungsphase*.” According to our experience, the phase of secretory activity reaches its height within about one hour and then slowly diminishes, whereas the storing-up phase reaches its height only after twelve to twenty-four hours. From this alone one can conclude that the storing-up process is independent of the secretory process or of the intensity of the secretion. On the contrary, at that time when the cell is being most intensely perfused with the dye fluid, the granules are only very weakly stained, and then gradually the intensity of the staining increases in spite of the progressive diminution of the secretion. Nevertheless, it seems that the storing up of carmin is more or less dependent on a certain optimum rapidity of the intracellular perfusion. Increasing the rapidity of excretion by means of a diuretic immediately results in a prolongation of the period of storing up. Lowering its rapidity, as, for example, by lowering the blood-pressure, results similarly in a delaying of the storing-up process. A faulty staining of the granules by the vital stains may therefore be the result of either of two diametrically opposed functional disturbances, a hyperfunction or a hypofunction.



We, therefore, arrive at the conclusion that the vital staining of the granules in the renal epithelium has absolutely nothing to do with the actual secretion itself. The prolonged perfusion of the epithelial cells with the dyestuff merely offers the granules the possibility of storing it up. The intensity of the granule staining cannot, therefore, be used as an estimate of functional capacity, as has until now been done, or at best only with very careful reservations. I should like to emphasize that point most emphatically, for even in the most recent work the opinion has been repeatedly expressed that the intensity of the granule staining stands in direct proportional relationship to the functional capacity of the cells. In making such statements the normal physiological differences in intensity with which the dyestuff is stored up in the cell granules of the different divisions of the *Hauptstück* have, of course, been left entirely out of consideration.

On the other hand, the fact that the storing up of the vital stain occurs in definitely characteristic fashion in each of the different divisions of the "*Hauptstück*," enables us to solve the important question whether the various renal poisons (cantharidin, arsenic, chromium, uranium, sublimate, etc.) damage the entire "*Hauptstück*" diffusely, or whether they possess a selective action on certain portions. Here again it is impossible for me to go into details. I desire merely to sum up the matter very briefly by saying that such a selective action does actually occur.

The various renal poisons permit a classification into three groups.

1. Those which damage mainly the terminal portion of the *Hauptstück*, but whose action may under the influence of larger doses also extend somewhat upward on to the middle division of the *Hauptstück*. Very rarely and only when extremely large doses are administered will the action extend on to the proximal division of the *Hauptstück* as well. To this group belong sublimate and cantharidin.

2. Those in which the damaged area is chiefly limited to the spiral portion of the distal division of the *Hauptstück*, spreading downward toward the Henle loops and upward on to the middle division, but rarely reaching as high as the proximal division. Characteristic of this group of poisons is uranium.

3. Those which primarily damage the proximal and middle divisions of the *Hauptstück*, though with larger doses spreading downward on the distal and transitional divisions. To this group belongs chiefly chromium.

I should not lay any stress on this specific localization of the action of the various renal poisons, were I not convinced that it possesses an important bearing on the analysis of the function of the kidney. It certainly cannot be an indifferent matter which of the three or four divisions is chiefly damaged by a certain poison, particularly as we know

absolutely nothing at present concerning what special functions each of these divisions may possess. In fact, on the basis of our numerous series of experiments on the artificial production of hydronephrosis, the injection of uric acid and the observation of physiological forms of epithelial pigmentation (H. Fischer), I believe we have the right to conclude that the various divisions differ from one another in regard to their excretory function.

In this regard I can to-day only refer you to the more detailed publication. I should merely like to mention that uric acid is mainly excreted in the first and second divisions of the *Hauptstück* and that the lipid substances, as, for example, in diabetes, are similarly stored up in these divisions. On the other hand, Baehr of New York, when working in my institute, was able to demonstrate that in the diabetic kidney glycogen is not only stored up intracellularly, but also excreted specifically by the cells of the fourth or transitional divisions of the *Hauptstück*. The work proved that the glycogen deposition in the diabetic kidney is not an incidental degenerative process, situated, as has previously been thought, in the Henle loops. On the contrary, it is an important evidence of a specifically localized secretory process. Of especial interest is the fact, to which Baehr has also called attention, that cantharidin, which acts specifically on this fourth or transitional division, is able to render the kidney relatively impermeable to sugar.

The question concerning the differences in action of the poisons is made still more complicated by the fact that they not only pick out particular portions of the *Hauptstück*, but that they also produce different types of damage. For example, chromium, uranium and sublimate produce extensive epithelial necroses in the affected tubules, whereas cantharidin chiefly produces a very marked cellular swelling and vacuolization. And then, finally, even the necroses following the various poisons show differences, in that they may or may not be preceded by a stage of hyalin-droplet degeneration. These facts possess an important bearing on the formation of casts. For in poisonings with those substances which produce the cellular necroses, casts are present in large numbers, whereas in cantharidin and arsenic poisoning they are relatively infrequent. If it were true that cantharidin particularly damages the glomeruli, as most authors have assumed, then the formation of casts following its administration should, according to the teaching of Ribbert, be especially marked. In our numerous experiments with cantharidin we have practically never observed any damage to the glomeruli which was worthy of mention, or which could not similarly be observed following any of the other poisons. We cannot, therefore, agree with those authors who are of the opinion that a glomerulo-nephritis, or a specific damage to the glomerular capillaries of the renal vessels can be produced by the

use of cantharidin. Our experiments with arsenic have also led to a negative result in this regard.

The actual starting point of our researches along these lines was the work of Schlayer and Hedinger on toxic nephritis several years ago. On the basis of carefully planned experiments, these authors recommended a sharp distinction between vascular and tubular nephritis, which seemed to us to be worthy of a careful histological analysis. We were able to convince ourselves that, on the one hand, the topographical relationship of the damage effected by the different renal poisons employed by these authors was exceedingly varied and complicated; that, on the other hand, the histological picture following cantharidin poisoning showed that with far more justice it deserved to be classed as a tubular poison rather than a vascular, and that finally the great differences in blood-pressure which the authors themselves admit to be present in the tubular and the so-called vascular poisoning is more than sufficient to account for the existing differences in function. For these reasons, it seems to us that the division of the renal poisons into a tubular and a vascular group is not justifiable. We have been forced to the conclusion that all the poisons which we have studied act primarily on the parenchyma, and in fact on particular portions of the *Hauptstücke*. The variations in the functional disturbance produced by certain of the poisons can be better explained by differences in the topography of the area affected, and by the fact that the individual poisons have a different influence on the blood-pressure.

The knowledge that all the known renal poisons as at present administered act practically exclusively, in so far as is morphologically demonstrable, on the epithelium of the *Hauptstücke*, destroys all hope of producing experimentally by this means pathological pictures resembling the human nephropathies. Of course, contributions are accumulating in the literature which prove that if administered over a long enough period even these parenchymatous poisons may produce a contracted kidney. But for man it is, on the contrary, just the diseases of the glomeruli which form the most important etiological factor for contracted kidney. Sclerotic areas in the kidney have been produced by Ophüls with lead and with chromium, by Dickson with uranium, by Uyeno under Ponfick's direction with carbolic acid, and the question is still to be settled how these foci of sclerosis are to be explained. One author assumes a direct irritation to the connective tissue, the proliferation of which eventually compresses the epithelial elements. On the other hand, Uyeno and Ponfick believe that the damming up of the tubules by means of casts is the primary cause of the sclerotic areas; that the proliferation occurs in the vicinity of these obstructed tubules, and that this connective tissue increase by gradual contraction and compression causes the tubular shrinkage.

The pathological pictures which we obtained in chronic uranium poisoning thoroughly convinced us that neither of these two hypotheses represent the real causative factor, although it cannot be denied that the retention of casts is of some significance. The great majority of the casts, however, are flushed out of the organ in a relatively short time and only occasionally may some remain behind, become calcified and thereby incite a reactive inflammation in its vicinity which may lead to total obliteration of the lumen. We feel convinced that still another process plays the chief rôle. In accordance with the work of the most recent authors, amongst which that of Thorel, of Heinecke and of Tilp must be especially mentioned, we were able to observe the extraordinarily rapid epithelial regeneration in the damaged *Hauptstücke* in all animals, and could readily determine that this epithelialization had its point of origin primarily in the narrow limb of Henle's loop and in the remains of the lower transitional division of the *Hauptstück*. Should the advance of the proliferating epithelium be obstructed by impregnable necrotic masses, or the epithelium from which the regeneration ordinarily starts be too severely damaged, there then results a collapse, and, eventually, total destruction of that particular entire tubular system. The portion of the tubular system proximal to the point of obstruction undergoes a pressure and inactivity atrophy absolutely identical with that occurring in hydronephrosis. And in this manner the numerous atrophic islands in the renal cortex develop, which are particularly characterized by the fact that the glomerulus belonging to the atrophic focus is somewhat smaller than normal, but otherwise strikingly well preserved, that its capsule is thickened, and its capsular space dilated. Although the process I have just described leads to a tubular contracted kidney as distinguished from a glomerular, it nevertheless does in my opinion find its analogy in human pathology in the pyelonephritic contracted kidney, the uric acid kidney and the sclerotic foci proximal to renal cysts, etc.

But since the glomerular contracted kidney is, as a matter of fact, really the predominating type in human pathology, the most important problem was to find a means of producing a glomerulonephritis in animals. I have already mentioned that the glomerular lesions observed after the administration of the ordinary renal poisons can by no means be considered a genuine glomerulonephritis. Only in uranium nitrate poisoning have Christian and Mackenzie been able to observe certain changes in the glomerular loops which would seem to indicate a possible vulnerability of the glomeruli in regard to uranium.

Baehr has recently been studying these various problems in the Freiburg Institute in greater detail. By the subcutaneous, but above all by the intra-arterial administration of uranium, he has succeeded in producing experimentally absolutely typical pictures of human glomerulo-



nephritis, and especially of the Löhlein type of glomerular lesion occurring with chronic ulcerative thromboendocarditis. The process begins with blood platelet thrombi in the capillary loops, necroses of the endothelial capillary walls and exudation into the capsular space. Then follows absolutely characteristic proliferation of the epithelium of Bowman's capsule with the formation of typical epithelial crescents. And, finally, there result adhesions which lead to partial obliteration of the capsular space. Hemorrhages also accompany the glomerular changes, so that even macroscopically the characteristic red punctate dotting of the cortex is to be observed. Unfortunately thus far the animals have usually died within the first few weeks, so that no very far-advanced stage of contracted kidney has as yet been obtained. But Baehr has for the first time produced glomerulonephritis experimentally, and his researches are the first which point the way to the experimental production of glomerular contracted kidneys. Of particular interest is the fact that by similar intra-arterial injection of iodine, chromium and croton oil no such glomerular changes could be produced, so that uranium must possess a specific action.

Damage to the glomerular apparatus must by no means be considered as identical with damage to the renal vessels, as some authors have wrongly assumed, and there have also been attempts to injure only the vessels themselves. As I have already mentioned, it was especially Schlayer who believed that in cantharidin and arsenic he possessed poisons which specifically damage the renal vessels. But as Pearce, Hill and Eisenbrey have shown, an absolutely sharp distinction between vascular and tubular poisons cannot be made. Furthermore, a critical study of Schlayer's experiments, which Baehr has recently undertaken, has also shown that among other things the general circulatory disturbance produced by the so-called vascular poisons is one of the chief causes of the early oliguria and other functional changes on which this classification is based. We possess at present no means with which to damage the renal vessels alone, and, therefore, no means with which to induce in animals a reproduction of the vascular type of contracted kidney.

It must, therefore, be frankly recognized that up to the present time only tubular contracted kidneys have been experimentally produced in animals, but that on the basis of Baehr's experiments we can also expect to produce glomerular contracted kidneys in the near future. Only then will it be possible to study satisfactorily the effect of such renal changes on the general organism, and, what will interest the clinician most of all, their influence on the vascular system, on the heart, and on the adrenals.

For the time being we are, therefore, obliged to confine ourselves to the pathological findings in our human material. But even in human pathology there exists up to the present time such a confusion in terminology and in interpretation that a satisfactory understanding of kidney diseases is a very difficult matter. This is the reason for the oft-repeated complaint that we possess no clear and reliable classification of renal diseases, and that we make no sharp distinction between the actual inflammatory processes and the other pathological disturbances which affect the kidney. It was no less a man than Friedrich Müller who, at the Meran Congress of the "*Deutschen Naturforscher und Aerzte*," distinctly advocated a division of the non-inflammatory from the inflammatory kidney affections under the names of the "Nephroses" and the "Nephritides," respectively. But the term nephrosis has not come into general use, and in order to preserve a terminology analogous to that for diseases of other organs, it would be better to replace it by the term "Nephropathy."<sup>1</sup>

Inasmuch as we know so very little concerning the etiological factors, it is permissible to classify the nephropathies on the basis of pathogenesis. We can best divide all diseased conditions of the kidney into those which are purely or mainly passive in nature, and those which are reactive or inflammatory in nature. The first group can be again divided into the disturbances in development and form, the disturbances in circulation and the metabolic disturbances; the second group, which is really a complicated mixture of passive and active processes, must also be arranged into subdivisions according to whether the inflammatory reaction chiefly limits itself to the blood-vessels and connective tissue, or to the glomerular apparatus or to the tubular apparatus with its own individual divisions.

Finally, according to the course of the pathological process, some of the subdivisions must be separated into the acute and the chronic affections. Although it is extremely tempting to compare for you the acute renal diseases with those facts which have been determined experimentally in animals, I nevertheless feel that I must confine myself to a brief sketch of the chronic renal affections, because, in default of material for satisfactory comparison in the field of experimental work, such a brief outline seems more necessary for a better understanding of the present-day conceptions. I have attempted to present a survey of the chronic renal affections alone, at the same time indicating the acute processes from which these develop. Although in the chronic affections of mainly passive origin — those of formative, degenerative or circulatory origin — the pathological condition under consideration usually develops

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1. As used by me in 1909, and recently recommended by Barker (*Am. Jour. Med. Sc.*, 1913, cxlv, 42).

insidiously from the very onset; the chronic affections of inflammatory origin are, on the contrary, usually ushered in by an acute process. The various forms of inflammatory contracted kidney result secondarily therefrom, and ought really to be considered as nothing more or less than cicatrices in various stages of development. In fact, the inflammatory contracted kidneys resemble the other chronic nephropathies of passive nature in this respect, for the reactive process is more or less completely extinguished and only a physical disproportion and perhaps a certain disturbance in functional equilibrium remains.

It is not my purpose to enter on a detailed description of these various chronic renal affections, referring you rather to the text-books of pathological anatomy in which the frequent and confused misunderstandings, as, for example, that in regard to chronic parenchymatous nephritis, have been subjected to a searching exposition in the light of recent investigations. But I desire to call your attention to one point, namely, the great variety which the several forms of contracted kidney present in regard to their pathogenesis, although they form only one subdivision of the chronic renal affections. I shall limit myself to a comparison of the genuine and the secondary contracted kidney.

#### THE CHRONIC NEPHROPATHIES

##### I. Nephropathies of *formative* origin (disturbances in development and form).

- Examples: 1. Nephropathia cystica. Congenital cystic kidneys.  
 2. Nephropathia hydronephrotica. Hydronephrotic contracted kidneys.  
 3. Nephropathia carcinomatosa, etc. Carcinoma of the kidney, etc.

##### II. Nephropathies of *degenerative* origin (metabolic disturbances).

- Examples: 1. Nephropathia diabetica. Glycogen excretion and deposition in the lowermost division of the "Hauptstück" (convoluted tubule of first order).  
 2. Nephropathia urica. Uric acid collections in the resorbing portions of the renal tubules.  
 3. Nephropathia amyloidea. Amyloid degeneration of the capillary system.  
 4. Nephropathia Basedowiana. Fat infiltrations in the secretory portions of the renal tubules.

##### III. Nephropathies of *vascular* origin (circulatory disturbances).

- Examples: 1. Nephropathia albuminurica orthostatica adolescentium.  
 2. Nephrosclerosis cyanotica. Kidney of passive congestion.  
 3. Nephrocirrhosis embolica. Embolic contracted kidney.  
 4. Nephrocirrhosis arteriosclerotica. Arteriosclerotic contracted kidney.  
 5. Nephrocirrhosis genuina. Genuine or primary contracted kidney. Granular atrophy. Presenile sclerosis affecting the smallest branches of the renal artery.

IV. Nephropathies of *inflammatory* origin.

- Examples: 1. Nephrocirrhosis glomerularis. Glomerular or secondary contracted kidney. (The "chronic parenchymatous kidney" of older authors). Develops after an acute glomerulonephritis.
2. Nephrocirrhosis tubularis. Tubular contracted kidney. Develops after an acute tubular nephritis. Exceedingly rare in man, if it occurs at all. In animals it can be experimentally produced by chronic uranium poisoning, and by intra-arterial injection of iodin.
3. Nephrocirrhosis apostematica. Pyelonephritic contracted kidney. Develops after an acute suppurative inflammation of either hematogenous or urinogenic origin.
4. Nephrocirrhosis tuberculosa, etc. Tuberculous contracted kidneys, syphilitic, etc.

Even at the present day the idea is still widespread that the genuine or primary contracted kidney is the result of an inflammatory process, and the possibility cannot be denied that such a mode of origin may occasionally occur, as, for example, following the acute exudative lymphocytic or leukocytic nephritides of scarlet fever and of streptococcus infection, a possibility which even I myself have mentioned in my textbook. But it is now, as a matter of fact, beyond the shadow of a doubt that the majority of the uncomplicated cases develop as a result of a vascular disturbance, or, to be more exact, from a sclerosis of the finest branches of the renal artery, beginning relatively early in life and leading secondarily to the formation of atrophic foci in the parenchyma and to hyaline degeneration of the glomeruli, with a resultant focal inactivity atrophy of the tubules belonging to them. These facts, originally brought forward by Jores, have been thoroughly confirmed by Loehlein, Herxheimer, Fahr and Gaskell. This is, therefore, no primary disease of the filter apparatus; that is, of the glomeruli, but of the blood-vessels leading to the filter, a presenile arteriosclerosis of the finest branches of the renal artery.

The old teaching of Gull and Sutton concerning the arteriocapillary fibrosis in the "red contracted kidney" is herewith again brought to the front and properly honored. But, indeed, what induces primary disease of the smallest arterioles is still an unsolved problem. Is it only one manifestation of a general disease of all the smallest arteries (of the pancreas, retina, brain, etc.)? Or does it precede all the others and only secondarily, by reducing the volume of the afferent flow of blood to the renal filter, either reflexly or chemically call forth a constant high blood-pressure with its consequences, the classical cardiac hypertrophy and the sclerosis of the peripheral arterioles due to overwork and overuse? And if it does precede, to what is it then due? Is it the result of a congenital inferiority of the renal vascular apparatus, or is perhaps the filter relatively too small and its total diameter too narrow for the volume of its afferent blood-current? Is it perhaps due to some specific effect of



the ingested food or of endogenous irritants on the afferent blood-vessels of the renal filter? These are the problems which are still waiting for solution, as is also the question concerning the relation of the entire process to the chromaffin system. Only one thing seems certain, and that is that the renal sclerosis and contraction is primarily passive in nature, the result of the disease of the renal vessels. In this respect, this type of contract kidney approaches the arteriosclerotic, in which the disease, as one might say, spreads from the aorta outward into the individual branches of the renal artery and so only induces an inactivity atrophy of isolated, but larger areas of the kidney parenchyma.

Nevertheless, the amount of the filter which is incapacitated because of the peripheral disease of the renal vascular system in the genuine contracted kidney is far greater than that in the arteriosclerotic, and for this reason the latter does not induce such an outspoken secondary effect on the general vascular system and the heart. The larger the amount of filter, that is, of glomerular tissue functionally incapacitated, the greater must be the circulation through the remaining filter tissue in regard to blood-pressure and speed of the current, in order to keep up the amount of excretion necessary for the organism. This remaining filter tissue works on the borderline of its maximum functional capacity as long as its blood-supply is maintained at the proper speed and under a sufficiently high pressure. Hence, the normal output of urinary fluid and even polyuria. But it must be borne in mind, that although the kidney performs an increased work output, similar to that in cardiac hypertrophy, this always remains within definite limits and can, indeed, be chiefly increased by influencing the heart. This accounts for the relatively limited power of accommodation in the polyuric vascular contracted kidneys. Even the tubules that remain undamaged work more or less at their maximum. This interpretation of the process at least permits us to explain the frequent, and often very marked compensatory hypertrophy of the remaining glomeruli, as well as the remaining tubules, in such contracted kidneys.

The problem of the secondary contracted kidney is an entirely different matter; the primary factor is here an inflammatory change affecting the renal filter apparatus itself. The significance of this initial glomerular disease, attention to which had previously been called by Nauwerk, v. Kahlden, Ribbert, Reichel, etc., has recently again been particularly emphasized by Loehlein; and the subsequent studies of Gaskell, Bachr and others, have supplied ample confirmation of its importance. But these inflammatory contracted kidneys do not alone differ from the genuine vascular contracted kidneys in that the primary seat of the disease is in the glomeruli. One of their chief characteristics is usually the *general* involvement of the entire filter apparatus by the

pathological change, so that the reparative and the regenerative compensatory processes find much more difficulty in effecting a restoration of the normal function. For this reason the slightest overexertion, or even the physiological variations in renal work may very easily cause the renal tissue to quit work, thereby inducing uremia and the characteristic edema. The clinical picture is usually dominated by a glomerular oliguria, although if a sufficiently large part of the filter has recovered, this may give way to a polyuria. And this polyuria, exactly like that accompanying the genuine contracted kidney, depends on overwork of the remaining normal portions of the filter apparatus under the influence

TABLE SHOWING LOCALIZATION OF THE ACTION OF VARIOUS TOXIC AGENTS ON THE URINIFEROUS TUBULES OF THE RABBIT'S KIDNEY

Portions Generally Participating	Kind of Poison	Portions Participating to a Less Extent
Proximal Convoluted Tubule (Hauptstücke)	Chromium ..... Uranium .....	
Section 1.....	Cantharidin ..... Sublimate .....	
Section 2.....	<div style="text-align: center;"> <math>\downarrow</math> <math>\uparrow</math> </div> Uranium .....	Ascending limb of loop and intercalary piece.
Section 3.....	Cantharidin ..... Chromium ..... Uranium .....	
	Sublimate .....	Intercalary piece and ascending limb of loop.
Transitional Section.....	Cantharidin .....  Chromium ..... Sublimate .....	Ascending limb of loop and intercalary piece.

of an increased circulation through them, combined with an increased blood-pressure in the entire general vascular system. All our functional tests will actually test only these remaining normal portions of the filter apparatus which are working more or less at their maximum, and their particular vessels; but it must be remembered that they do not test the diseased tissue which is now in the scar stage.

Here in the secondary contracted kidney, the tubular secretory tissue connected with each diseased glomerulus is similarly put out of function, just as in the genuine contracted kidney, but usually to an even far more widespread extent.

The above considerations indicate that in the severe forms of nephritis oliguria depends on a glomerular disease and not on a disease of the

blood-vessels. Also the polyuria, both in the nephritic as well as in the vascular contracted kidney, in so far as the histological findings seem to indicate, does not depend on a hypersensitiveness of the still diseased vessels, but on the increased work of the undamaged vessels and their respective filter apparatus, or the only slightly damaged ones, or those which have entirely recovered from their damage.

These forms of polyuria, which I might term compensatory or accommodative in nature, must be sharply distinguished from the acute irritative polyurias of toxic origin. The latter depend on a local damage to the blood-vessel filter apparatus, the former on a functional accommodation, usually accompanied by simultaneous accommodative processes in the rest of the general vascular system.

I hope that with these few examples I have made it clear that the problem of acute nephritis is entirely different from that of the chronic nephropathies, just as in toxicology the problem of acute poisonings differs from that of the chronic. In the acute nephritides we have to do with the question of an acute damage to previously normal functions; in the chronic nephropathies the question is that of the possibilities for compensation and of a failure of these compensatory regulations. The opportunity to penetrate more deeply into these two problems and to bring forth the solution has now been made possible by the recent advances in our knowledge concerning the normal structure of the organ and the experimental production of tubular and glomerular contracted kidneys.

# STUDIES ON URIC ACID OF BLOOD AND URINE, WITH SPECIAL REFERENCE TO THE INFLUENCE OF ATOPHAN \*

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Until Folin devised his recent colorimetric method for quantitatively determining the uric acid of the blood, we were very much in the dark as to the actual amount of this substance in circulation. Abderhalden, in his text-book, states that the amount of uric acid in the blood is too minute for determination. The older methods, the cupric bisulphite method of Krüger and the silver nitrate method of Salkowski, are too crude and require too large an amount of blood for practical service. Folin hints that Garrod must have drawn considerably on his imagination in his "*Thread*" estimates, and expresses the opinion that practically all of the older statements in the literature as to the amount of uric acid in the blood, represent nothing more than guess-work.

This new method possesses two essentials: it is remarkably accurate and it requires comparatively small quantities of blood — from 15 to 25 grams.

Briefly, uric acid is precipitated from the mother liquor of a weighed amount of blood, and is again brought into solution in very small volume. The uric acid thus obtained is treated with Folin's phosphotungstic acid reagent and gives a beautiful blue color which can be compared in a colorimeter with a known standard uric acid solution. I have found the Autenreith-Königsberger colorimeter (in common use in the pthalein test of kidney function) admirably adapted to this purpose.

The accuracy of Folin's method can be tested by adding, as he has done, a weighed amount of uric acid to sheep's blood (which contains a negligible quantity of this substance) and then proceeding as with human blood.

## I

Folin's determinations of uric acid in the blood of unselected individuals on a mixed diet is the basis of the first accurate statements in this regard. His figures run from 0.7 mg. to 3.7 mg. of uric acid in 100 gm. of blood, the overwhelmingly larger number of cases giving values from 1.0 mg. to 2.0 mg.

I wish to report a number of estimations on the blood of unselected healthy individuals on a purin-free diet. I have also made similar

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\* Submitted for publication Aug. 20, 1913.

\* Read in the Section on Practice of Medicine of the American Medical Association, at the Sixty-fourth Annual Session, held at Minneapolis, June, 1913.



determinations in unselected cases of various diseases. With one exception, to which attention will be called, the individual was kept on a purin-free diet at least three days before observations were begun.

TABLE 1.—URIC ACID ESTIMATION IN NORMAL INDIVIDUALS

Mg. Uric Acid in 100 Gm. Blood			
J. C.....	0.5	R. D.....	1.3
H. D.....	0.6	L. S.....	1.4
M. D.....	0.6	L. H.....	1.7
A. B.....	0.8	R. O.....	2.1
R. C.....	0.9	H. H.....	2.5
H. D.....	0.9	J. M.....	2.5
J. G.....	0.9	R. E.....	2.9
S. M.....	1.1		

Wide variations are seen in Table 1. The specimens of blood showing the smallest amounts of uric acid were taken from medical students leading rather sedentary lives. The three highest were from professional men; the highest from a very active civil engineer.

TABLE 2.—VARIATIONS IN URIC ACID IN A SINGLE INDIVIDUAL

Blood		24-Hour Urine			
Mg. of U.* in 100 Gm.		Amt. c.c.	U. Gm.	NH <sub>3</sub> Gm. N	N. Gm.
3/20 (Lost)		1,375	.44	.36	13.86
4/ 2 2.3		1,700	.34	.62	12.58
4/ 8 2.5		1,650	.29	...	9.02
5/21 2.4		1,410	.39	...	10.37
5/27 2.4		1,660	.31	.66	11.19

\* U. in this and following tables means uric acid.

It has been known for a long time that while different individuals excrete in the twenty-four hours varying amounts of uric acid, such elimination for one and the same person is fairly constant. To ascertain whether the blood of a given individual on a purin free diet maintains at all times a constant uric acid content, the determinations of Table 2 were made. It appears here that while the uric acid of the urine varies to some extent, that of the blood is remarkably constant.

TABLE 3.—URIC ACID OF BLOOD IN DISEASE

Mg. in 100 Gm. Blood			
Mitral lesion .....	0.5	Acute tuberculous pleurisy.....	1.7
Rheumatic fever .....	0.6	Chr. Interst. nephritis.....	1.8
Acute syphilis .....	0.8	Malaria .....	1.8
Chr. tuberculous pleurisy.....	0.8	Pneumonia .....	1.9
Pneumonia .....	1.2	Uremia .....	2.1
Pneumonia .....	1.3	Polycythemia .....	2.2
Sciatica .....	1.3	Graves' disease .....	2.5
Typhoid fever .....	1.4	Pneumonia .....	2.7
Ulcer of stomach.....	1.5	Gout .....	3.3
Pneumonia .....	1.6	Arterial hypertension .....	3.3
Amebic dysentery .....	1.6	Intermittent gastric supersecretion	3.7
Neurasthenia (?) .....	1.7	Gout .....	4.5

Determinations were made on the blood of a number of unselected patients suffering from various diseases. While rather wide variations in

Table 3 are seen, it is noted that gout, as has been pointed out by other observers, gives the highest values. Whether or not the patient showing the second highest amount of uric acid was a sufferer from the so-called gouty diathesis, is an interesting question. His disease has been called intermittent gastric supersecretion for want of a better diagnosis.

## II

Much discussion has been devoted to atophan (2-phenylchinolin-4-carbonic acid), a drug devised by Nicolaier and Dohrn, and claimed by them to markedly influence purin metabolism. Numerous uric acid determinations with reference to the influence of atophan on the urine have been made by other observers. All agree that this drug increases the uric acid of the urine. Various theories as to its manner of action have been expressed. Nicolaier and Dohrn believe that atophan influences, in some way, purin metabolism within the muscle and thus leads to increased formation and excretion of uric acid. So far as I know, no determinations of the blood uric acid, as influenced by atophan, have been made. The accompanying tables detail certain changes noted in the uric acid of the blood and urine in health and disease following the administration of atophan.

TABLE 4.—THE INFLUENCE ON URIC ACID IN BLOOD AND URINE OF ATOPHAN IN HEALTH

Date		Blood Mg. U. in 100 Gm.	Twenty-Four Hour Urine			
			Amt. c.c.	U. Gm.	NH <sub>3</sub> Gm. N.	N. Gm.
3/20	.....	...	1,375	.44	.36	13.86
3/21	A .....	...	2,240	.57	.42	.....
4/ 2	.....	2.3	1,700	.34	.62	14.04
4/ 3	.....	...	1,550	.35	.65	12.58
4/ 4	A .....	1.6	2,450	.55	.73	14.03
4/ 8	.....	2.5	1,650	.29	...	9.02
4/10	A .....	2.1	2,333	.35	...	15.54
5/21	.....	2.4	1,410	.39	...	10.37
5/22	A .....	1.8	1,850	.72	...	13.20

In Table 4 the letter "A" indicates the day on which atophan was given. Two grams of the drug were administered in one dose and the blood obtained two hours later. Following the administration of atophan quite a drop in the uric acid of the blood occurs; simultaneously an increase in the total amount of urine with marked increase in its uric acid is seen. A slight increase in the ammonia and the total nitrogen of the urine also follows. The increase in the total nitrogen, however, does

not keep pace with the increase in the uric acid, and I think we are justified in concluding that atophan exerts a selective influence on the elimination of the latter.

TABLE 5.—EFFECT OF ATOPHAN ON EXOGENOUS PURINS

Date	R. E.	Blood Mg. U. in 100 Gm.	Twenty-Four Hour Urine			
			Amt. c.c.	U. Gm.	NH <sub>3</sub> Gm. N.	N. Gm.
5/28	.....	2.9	1,150	.46	.58	10.26
5/29	7 a. m. 500 Gm. Thymus	3.2	900	.66	.69	10.96
	2 p. m. Blood					
	7 a. m. 500 Gm. Thymus					
5/31	9 a. m. Atophan	1.1	1,280	.75	.76	11.16
	2 p. m. Blood					

In the observations recorded in Table 5 the effect of atophan on exogenous purins is noted. It is seen that the same decrease in the uric acid of the blood, and increase in that of the urine, occurs, as well as the same changes in amount, ammonia and nitrogen of the urine as noted in Table 4.

TABLE 6.—THE INFLUENCE OF ATOPHAN ON URIC ACID AND URINE IN DISEASE

Date		Blood Mg. U. in 100 Gm.	Urine			
			Amt. c.c.	U. Gm.	NH <sub>3</sub> Gm. N.	N. Gm.
5/24	Sciatica .....	1.3	.....	...	...	.....
5/26	A, Sciatica.....	0.9	.....	...	...	.....
5/20	Intmt. gstr. supersern..	3.7	880	.39	.74	6.77
5/23	A, Intmt. gastr. super- sern. ....	2.1	1,150	.41	.82	7.20
5/12	Aneurysm .....	1.0	.....	...	...	.....
5/14	A, Aneurysm .....	0.5	.....	...	...	.....
5/ 9	Chronic interst. nephrr..	1.8	.....	...	...	.....
5/10	A, Chr. interst. nephrr..	1.3	.....	...	...	.....
5/24	A, Cont. ....	1.5	.....	...	...	.....
6/ 4	A, Cont. ....	1.4	.....	...	...	.....
5/16	Gout .....	4.5	845	.32	.19	4.17
5/17	A, Gout .....	3.2	1,225	.46	.32	5.25
5/31	Gout .....	3.3	1,125	.33	.33	8.28
6/ 1	A, Gout .....	2.8	1,550	.42	.41	9.90
5/26	Graves' disease.....	2.5	1,300	.36	...	10.08
5/27	A, Graves' disease.....	1.6	750?	.33	...	8.40?

In Table 6 the effect of atophan on the uric acid of the blood and urine of patients suffering with various diseases is seen. Its influence seems to be the same as in health.

Clinically, the drug produces in gout very distinct amelioration of symptoms and decreases the size of the tophi. In other diseases, notably in one case of sciatica, in two of chronic arthritis and in two of acute rheumatic fever, no relief from pain or other amelioration of symptoms was noted.

TABLE 7.—COMPARATIVE DETERMINATIONS OF URIC ACID

Date	Blood Mg. U. in 100 Gm.	Time	Urine			
			Amt. c.c.	U. Gm.	NH <sub>3</sub> Gm. N.	N. Gm.
5/27	...	9 a. m.	(365)	(.058)	(.087)	(2.01)
5/28	2.4	9 a. m.	420	.072	.099	1.47
5/27	...	12 m.	(250)	(.075)	(.066)	(1.68)
5/28	1.2	12 m.	780	.234	.122	2.73
5/27	...	3 p. m.	(405)	(.048)	(.093)	(1.79)
5/28	0.7	3 p. m.	105	.058	.080	1.02
5/27	...	6 p. m.	(200)	(.065)	(.082)	(1.66)
5/28	0.6	6 p. m.	65	.010	.069	.82
5/28	...	6 a. m.	(440)	(.167)	(.236)	(5.05)
5/29	...	6 a. m.	310	.170	.287	5.33

Table 7 represents certain comparative determinations which were made at three-hour intervals. On a given day, starting at 6:00 a. m., with an empty bladder, the urine was collected at three-hour intervals until 6:00 p. m., and again at 6:00 a. m. the following day. The figures in parentheses represent the determinations made on these specimens. The next day the same procedure was followed, with certain additions; 3 gm. of atophan was given at 9 a. m., and blood for uric acid determinations was obtained at this hour and every three hours thereafter until 6 p. m.

As a result of the administration of three grams of atophan the following occurred:

In three hours (9 a. m. to 12 m.) the amount of uric acid in the blood was cut in half; the amount of urine passed during this period was treble that of the same period on the previous day, and the uric acid simultaneously eliminated was more than trebled; the ammonia was doubled and the total nitrogen almost doubled. In the next three hours (12 m. to 3 p. m.) the uric acid of the blood was again cut in half, but the total amount of urine passed was very small and the uric acid of the urine very little more than on the previous day. The ammonia and the nitrogen were less.



In the next three-hour period (3 p. m. to 6 p. m.) the amount of uric acid in the blood remains the same; unusual oliguria appears; the uric acid of the urine is remarkably low, the ammonia is reduced and the total nitrogen very much reduced.

It would thus appear from Table 7 that atophan stimulates the kidney to unusual activity and causes the rapid elimination of a large part of the blood uric acid. This stimulation seems to be followed by a reaction, in which a lessened amount of work is done by the kidney, the lowest ebb of its physiologic activity being reached about nine hours after the administration of the drug.

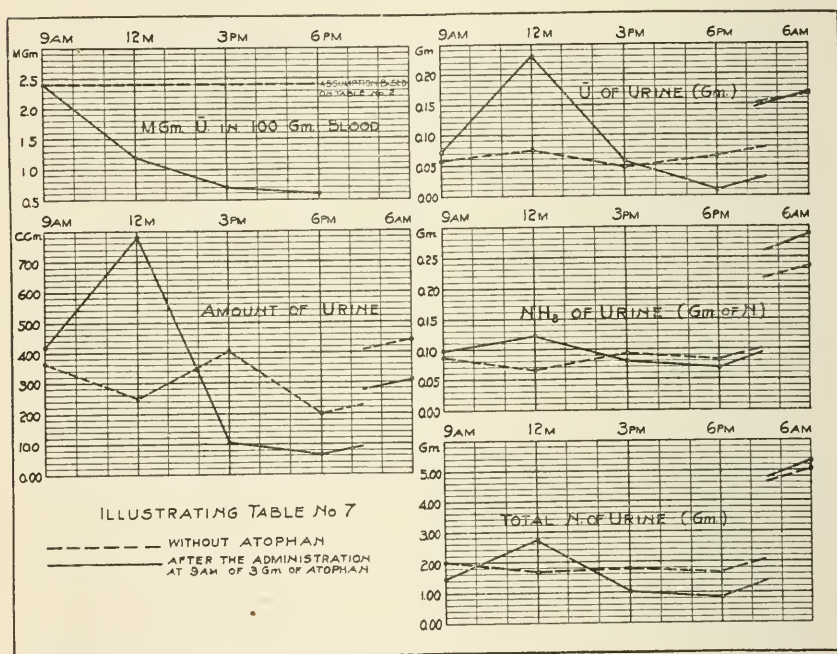


Chart showing comparative amounts of uric acid,  $\text{NH}_3$  nitrogen and total nitrogen in blood and urine before and after the administration of atophan. Dotted curve, without atophan; solid line after administration of 3 gm. of the drug.

#### CONCLUSIONS

1. The amount of endogenous uric acid in the blood of different individuals varies widely; yet, for a single individual, this figure is a constant one.

2. (a) Atophan increases general renal activity; in addition, it exerts a selective stimulating influence on uric acid excretion. (b) It simultaneously reduces the uric acid of the blood. (c) Its influence over uric

acid metabolism would seem to be limited to its power of transferring, by means of the kidney, uric acid from the blood to the urine.

NOTE.—Since the above was submitted for publication, Folin and Lyman have detailed in the *Journal of Pharmacy and Therapeutics*, 1913, iv, No. 6, similar experiments. They reach like conclusions regarding atophan, i. e.: "The results obtained in this patient furnish a striking illustration of the fact that phenyl-quinolin carbonic acid acts on the kidneys (Weintraud) and does not mobilize (Brugsch) deposited urates."

Empire Building.

SOME FORMS OF URINARY NITROGEN AFFECTED BY THE  
ADMINISTRATION OF DESICCATED THYROID  
TO DEMENTIA PRAECOX PATIENTS \*

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In a previous article, I showed that indol-acetic acid is frequently present in the urine of dementia praecox patients.<sup>1</sup> Later it was discovered that in the case of two dementia praecox patients the indol-acetic acid was endogenous and not derived directly from the tryptophan ingested.<sup>2</sup> Since there has been much discussion as to the relation of dementia praecox to the internal secretions and especially to that of the thyroid gland and since the administration of desiccated thyroid has given contradictory results as to its effect on nitrogen metabolism, it was thought worth while to determine the influence of the administration of thyroid primarily on the indol-acetic acid, and secondarily on the creatinin and total urinary nitrogen excretion.

PLAN OF TESTS

Four women patients, classified as cases of dementia praecox, were selected. They were all in apparently perfect physical condition. Their ages ranged from 29 to 32 years. Their approximate average weights were 94, 96, 115 and 101 pounds. During the test the patients were kept out of doors about six hours a day and were taken for a walk of nearly two miles daily.

The diet each day was constant in kind and quantity. Patients M. P. and M. O. B. received 6.3706 gm. of nitrogen daily during the first seven days of the test and 6.4995 gm. of nitrogen daily during the remainder of the time, in the form of coffee, milk, graham crackers, egg omelet and peanut butter. The daily diet of Patients G. G. and A. S. contained 9.2580 gm. of nitrogen and consisted of coffee, milk, graham crackers, a wheat breakfast food, ground meat and peanut butter.

The length of the feeding test was thirteen days. The second day the daily urine was collected and analyses begun. The eighth day a

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\* From the Illinois State Psychopathic Institute.

\* Submitted for publication Sept. 22, 1913.

1. Ross, Ellison L.: A Preliminary Note on the Excretion of Indol-Acetic Acid in the Urine, *THE ARCHIVES INT. MED.*, 1913, xii, 112.

2. Ross, Ellison L.: The Source of Urinary Indol-Acetic Acid in Two Dementia Praecox Patients, *THE ARCHIVES INT. MED.*, 1913, xii, 231.

5-grain tablet of desiccated thyroid was given each patient before each meal and this was continued for six days. The analyses were made before any of the urine was thirty-six hours old.

The Kjeldahl method was used for the total nitrogen determination, the Folin method for creatinin and my own method, slightly modified, for the indol-acetic acid. The method of determination of indol-acetic acid described in a previous paper<sup>2</sup> was modified, in that after adding the nitrite and hydrochloric acid, exactly three minutes were allowed to elapse before adding the amyl alcohol, and the shaking with the alcohol was shortened to one minute. The readings with the hemoglobinometer were begun exactly three minutes after the amyl alcohol was added.

The following Tables 1, 2, 3 and 4 give the results of analyses of the daily urines:

TABLE 1.—TOTAL URINARY NITROGEN EXCRETED PER DAY IN GRAMS

Days With and Without Thyroid	M. P.	M. O. B.	G. G.	A. S.
First without .....	2.7945	3.9379	5.9840	7.8285
Second without .....	4.9784	2.9917	6.1840	8.0530
Third without .....	5.1973	6.3867	5.4540	14.3170
Fourth without .....	6.3867	6.7932	6.6881	.....
Fifth without .....	5.3606	7.0635	6.3662	9.0680
Sixth without .....	6.0861	7.5744	7.5580	7.7110
First with .....	6.7718	6.5612	5.6615	7.7330
Second with .....	5.4859	4.3622	7.5710	9.3080
Third with .....	6.4445	10.6415	7.8365	9.0190
Fourth with .....	6.4294	7.3188	7.7660	12.3050
Fifth with .....	10.8638	6.9152	7.7800	9.1690
Sixth with .....	5.2370	6.5549	6.4150	6.4260

TABLE 2.—CREATININ EXCRETED PER DAY IN GRAMS

Days With and Without Thyroid	M. P.	M. O. B.	G. G.	A. S.
First without .....	0.3773	0.5918	0.5526	0.6801
Second without .....	0.6904	0.6046	0.6621	0.8892
Third without .....	0.7502	1.1097	0.7970	0.9788
Fourth without .....	0.8295	0.8532	0.8085	1.0480
Fifth without .....	0.6987	0.8826	0.6277	0.9668
Sixth without .....	0.7973	0.8664	0.6648	0.7778
First with .....	0.8413	0.7442	0.6528	1.0084
Second with .....	0.8466	0.5058	0.8564	0.9788
Third with .....	0.8562	1.2246	0.8599	1.0221
Fourth with .....	0.8223	0.7642	0.8108	0.8984
Fifth with .....	0.8850	0.8136	0.8100	0.8484
Sixth with .....	0.7320	0.8688	0.6102	0.8962



TABLE 3.—INDOL-ACETIC ACID EXCRETED PER DAY IN GRAMS

Days With and Without Thyroid	M. P.	M. O. B.	G. G.	A. S.
First without .....	0.0457	0.0550	0.1285	0.1912
Second without .....	0.1811	0.0606	0.1379	0.2291
Third without .....	0.2103	0.2875	0.1356	0.1888
Fourth without .....	0.2424	0.1968	0.1583	0.1962
Fifth without .....	0.1002	0.1603	0.2086	0.2528
Sixth without .....	0.1633	0.0662	0.1959	0.2361
First with .....	0.1838	0.0686	0.1382	0.5950
Second with .....	0.1372	0.0715	0.1550	0.2516
Third with .....	0.1997	0.1629	0.2125	0.2752
Fourth with .....	0.1548	0.1967	0.2631	0.2482
Fifth with .....	0.1723	0.1905	0.3533	0.3763
Sixth with .....	0.1442	0.2426	0.2489	0.4042

TABLE 4.—WEIGHT OF PATIENTS IN POUNDS

Days With and Without Thyroid	M. P.	M. O. B.	G. G.	A. S.
First without .....	....	....	115.00	100.75
Second without .....	....	....	114.00	100.75
Third without .....	94.50	96.52	115.00	100.75
Fourth without .....	....	....	114.75	100.50
Fifth without .....	94.25	96.00	114.00	100.50
Sixth without .....	....	....	114.00	100.25
First with .....	....	....	114.00	100.00
Second with .....	94.10	96.25	113.50	100.00
Third with .....	94.10	94.75	112.50	99.75
Fourth with .....	92.25	93.75	112.50	99.50
Fifth with .....	92.00	93.00	112.00	99.50
Sixth with .....	92.00	93.00	112.00	99.50

## RESULTS OF TESTS

Due to the fact that the entire cooperation of the insane patients could not be obtained, a sharp division of the urine produced in one day from that produced during the following day could not be made. This in part explains the irregularity of the daily results observed in Tables 1 to 4. Since the irregularities of the daily results make it difficult to see any general increase or decrease in the outputs, the average daily results for periods of three and six days (Tables 5, 6 and 7) have been calculated.

TABLE 5.—AVERAGE DAILY TOTAL URINARY NITROGEN EXCRETED PER PERIOD IN GRAMS

Days With and Without Thyroid	M. P.	M. O. B.	G. G.	A. S.
First three without.....	4.3234	4.4388	5.8740	10.0662
Second three without.....	5.9445	7.1437	6.8708	8.3895
Six without .....	5.1339	5.7612	6.3724	9.2278
First three with.....	6.2341	7.1883	7.0230	8.6866
Second three with.....	7.5101	6.9296	7.3203	9.3000
Six with .....	6.8721	7.0589	7.1716	8.9933

TABLE 6.—AVERAGE DAILY CREATININ EXCRETED PER PERIOD IN GRAMS

Days With and Without Thyroid	M. P.	M. O. B.	G. G.	A. S.
First three without.....	0.6060	0.7687	0.6706	0.8493
Second three without.....	0.7752	0.8674	0.7003	0.9309
Six without .....	0.6906	0.8180	0.6854	0.8901
First three with.....	0.8480	0.8249	0.7897	1.0031
Second three with.....	0.8131	0.8155	0.7437	0.8810
Six with .....	0.8304	0.8202	0.7667	0.9420

TABLE 7.—AVERAGE DAILY INDOL-ACETIC ACID EXCRETED IN GRAMS

Days With and Without Thyroid	M. P.	M. O. B.	G. G.	A. S.
First three without.....	0.1457	0.1344	0.1340	0.2030
Second three without.....	0.1690	0.1411	0.1876	0.2284
Six without .....	0.1572	0.1377	0.1608	0.2157
First three with.....	0.1736	0.1010	0.1686	0.3739
Second three with.....	0.1571	0.2099	0.2884	0.3429
Six with .....	0.1653	0.1555	0.2285	0.3584

## DISCUSSION OF RESULTS OF TESTS

Even the results for the three-day periods do not follow a regular trend in all cases, as is shown by Tables 5, 6 and 7. Not in all cases do both three-day periods of the same six-day period agree. A general increase in the outgo of the urinary nitrogen, creatinin, and indol-acetic acid, however, is shown in all four cases with one exception in the case of A. S. as regards the total urinary nitrogen. This patient was peculiar also in that the absolute quantities excreted were much larger than those excreted by any of the other patients and the daily variations were especially large.

The averages for the thyroidless and the thyroid period show distinctly the same increase. Table 8 will make this point clear.

TABLE 8.—RATIO OF THE ELIMINATIONS DURING THE PERIOD WITHOUT THYROID (TAKEN AS 1) TO THOSE DURING THE PERIOD WITH THYROID

	M. P.	M. O. B.	G. G.	A. S.
Total urinary nitrogen....	1.338	1.225	1.125	0.974
Creatinin .....	1.202	1.002	1.118	1.058
Indol-acetic acid .....	1.051	1.129	1.421	1.662

These results on the total urinary nitrogen agree with those of a number of investigators. The increase of the elimination of creatinin resulting from the administration of thyroid agrees with the results obtained by Pfeiffer and W. Schulz.<sup>3</sup>

Table 4 shows the gradual loss of weight of all of the patients during the test. This, according to the observations of many, would be expected. In these instances, however, the loss of weight cannot be said, with any certainty, to be due to the thyroid alone. It may have been due, partially or wholly, to partial inanition. The daily intake of protein by each patient, calculated as usual, was in the case of Patients M. P. and M. O. B., 39.8 gm. during part of the time and 40.6 during the remainder. In Patients G. G. and A. S. it was 57.9 gm. These amounts are not considered sufficient by most authorities. They were all that these patients could be induced to eat regularly, however, the weather being hot and oppressive.

The protein intake in these cases has an important bearing on the interpretation of the results on urinary nitrogen, creatinin and indol-acetic acid. It is well known that during inanition the total urinary nitrogen and the endogenous nitrogen is decreased. If these patients were in a state of partial fasting, this alone would tend to decrease the total urinary nitrogen and endogenous nitrogen. Therefore there is a possibility that the thyroid has exerted a much stronger influence than is apparent.

#### CONCLUSIONS

The administration of desiccated thyroid to these four patients with dementia praecox caused: (1) an increase in the output of total urinary nitrogen; (2) an increase in the output of creatinin; (3) an increase in the output of indol-acetic acid.

3. Deutsch. Arch. f. klin Med., 1899, lxi, 369.

## MORPHOLOGY OF THE BLOOD IN EPIDEMIC PAROTITIS \*

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Perhaps it is because of our general disregard of its importance as a disease that so little work has been done on the morphology of the blood in epidemic parotitis. From such a viewpoint it is true that a perfect knowledge of the pathological physiology of the disease is of comparative little import. But this disease, it has been observed, is accompanied by a lymphocytosis, and the problem of lymphocytosis is an interesting and important one. The blood-picture of this disease is also of distinct value in differential diagnosis.

A perusal of the literature on the blood-picture in this disease shows it to be very fragmentary; so that there is need for summarizing and a proper correlation of the results obtained by the various observers.

Since the work of Wile,<sup>1</sup> I have been unable to find any noteworthy contributions. Wile's bibliography refers to the work of Cabot,<sup>2</sup> who notes an absence of leukocytosis in this disease. Sasquépée<sup>3</sup> notes a slight leukocytosis, low polynuclears, increased mononuclears, a decreased eosinophil count and in complicating orchitis a leukocytosis. Krestnikow<sup>4</sup> records a lymphocytosis, mononuclear increase, and in complicating orchitis a polynuclear increase. Pick<sup>5</sup> found a leukopenia, increased mononuclears, decreased polynuclears and decreased eosinophils, in the orchitis a leukopenia. Turck<sup>6</sup> found a leukocytosis and eosinophilia. Wile found a lymphocytosis relative and absolute; eosinophils first lessened, later increased; polynuclears varying with the mononuclears. Complicating orchitis causing a tendency to polynuclear increase.

From these findings it appears that there is manifestly a lack of agreement among the observers as to what is the actual blood-picture. Some of the observers record a leukocytosis, while the others did not find it. A like difference of opinion exists with regard to the mononuclears and the eosinophils.

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\* Submitted for publication Oct. 21, 1913.

1. Wile: *Arch. Pediat.*, xxiii, 674.

2. Cabot: *Clinical Examination of the Blood*. (Ed. 5.)

3. Saquépée: *Arch. de méd. exper.*, 1902.

4. Krestnikow: *Thèse de St. Petersburg*, 1902.

5. Pick: *Wien. klin. Rundschau*, 1902.

6. Turck: *Verhalten des Blutes bei acuten Infections-Krankheiten*.



In this work, as in my work on pertussis,<sup>7</sup> I aimed to determine the successive changes in the leukocyte formula by studying the same cases throughout the disease, and after it had subsided, correlating these findings with the daily clinical notes.

#### MATERIALS

The eleven patients who come into this consideration were residents of the Industrial Home for Crippled Children, ranging in age from 9 to 20 years. Five of them were males.

Their general health had previously been good, and they were all free from such other disease activity at the time this epidemic appeared as might affect their cell counts.

#### PRECAUTIONS

The specimens were taken about an hour before meal time; thus avoiding a digestive leukocytosis. Another precaution was the counting of blood taken from both the lobe of the ear and the finger. On account of the proximity of the usual site for getting the specimens to the area of pathologic activity, it occurred to me that possibly the blood in such an hyperemic zone might be influenced more or less. But in a number of cases the blood-pictures were practically the same in both specimens.

#### METHOD

The first blood-count was made just as soon as the cases were suspected. At this time there was slight enlargement of the cervical and submaxillary lymph-nodes and perhaps a slight rise in temperature.

The second count was made on full development of the swelling, and the third when the temperature reached normal and the swelling was subsiding. Usually the temperature reached normal several days before the swelling subsided. After that, one or two counts were made at intervals of a week. \*

#### CONTROLS

It was impossible to get control counts prior to the onset of the disease, as there was no way of knowing who was exposed; but control counts were taken six months after the disease subsided when the children were in good health.

As a working formula for what should have been normal for these children, I assume that a leukocyte count of 8,000 and polynuclear of 72 per cent., lymphocytes 22 per cent., large mononuclears 3 per cent., transitionals 1 per cent., eosinophils 2 per cent., is about normal. If we count the lymphocytes and large mononuclears together, we have a mononuclear count of 25 per cent., and therefore 2,000 mononuclears to

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7. Barach, Joseph H.: Morphology of the Blood in Pertussis, *THE ARCHIVES INT. MED.*, 1908, i, 602.

the cubic millimeter. If a patient had 3,000 mononuclears, the figures read 50 per cent. increase and so on.

#### TOTAL LEUKOCYTE COUNTS

At the onset of the disease, when the patient is having mild constitutional symptoms and enlargement of submaxillary glands, but has not yet developed parotid swelling, the examination in two cases showed a leukopenia. One patient of 13 years showed a leukocyte count of 5,280 per c.mm., another of 20 years had 5,200 leukocytes.

During the height of the disease, when the parotid gland is swollen to its maximum, in the entire series the largest count was 7,800, the lowest was 4,000, the average being 5,700 per c.mm. This average is lower than my findings in a series of 150 cases of typhoid fever, in which leukopenia is one of the characteristic features. On studying the temperature charts and clinical notes in these cases I find that those which had the higher fever and greater parotid involvement showed the most marked leukopenia. In five cases in which the patients had most of the involvement on one side at the time of the examination, the count ranged between 6,100 and 7,800 per c.mm. In the remaining six, in whom both sides were equally large, and much larger than in the unilateral cases, the counts ranged between 5,000 and 5,680 per c.mm.

*Leukocyte Count When Swelling Recedes.*—By the time the parotid swelling was markedly diminished, which usually occurred between the fifth and seventh days of the disease, almost invariably the leukocyte count showed a cell increase.

*Leukocyte Counts After Swelling Subsided.*—In the counts made about a week after the swelling had subsided, the number of leukocytes seemed to vary, upward in half and down in the others. In the first half the leukocytes steadily increased toward the normal. In the others, their number oscillated.

*Leukocyte Count Six months Later.*—At this time when the children were all in their normal health, the leukocyte counts in every instance was considerably higher than during the activity of the disease. The average for the series being 7,920 per c.mm. as compared with 5,700 per c.mm. during the activity of the disease.

*Polynuclears.*—In not one case was there an absolute and relative increase of these cells throughout the attack. Two cases did show a relative increase in the early days of the disease.

In all cases as the parotitis subsided the polynuclears increased toward their normal number. Later counts showed irregularity as to the number of cells, but the counts were always higher than during the activity of the disease.

*Mononuclears.*—These were constantly above normal, except in two cases, which showed a decrease early in the disease.

The two exceptions behaved as follows: At the first three counts in one case there was a decrease in mononuclears of 5 per cent. to 2 per cent. and 10 per cent., while the three following counts showed an increase over normal of 33 per cent., 75 per cent. and 75 per cent.

In the second case, a young lady of 20, the mononuclears were decreased 25 per cent. and 5 per cent. at the first and second counts, but the third and fourth counts gave an increase of 5 per cent. and 30 per cent. in the mononuclears. These appear to be cases in which the blood reaction to the disease is delayed.

*The Eosinophils.*—Nearly all cases showed more of these cells after the disease had subsided than during its activity. But the total numbers and their proportions did not seem to present any definite course.

*Other Cells.*—Transitionals and mast-cells did not seem conspicuous in any of the counts. Nor did I notice any pathologic leukocytes, such as are occasionally found.

This series presented no opportunity for observing the effect of the complications of the disease on the blood formula.

#### SUMMARY

In uncomplicated cases of epidemic parotitis the leukocytes are affected in the following manner:

By the time the disease has manifested itself—even though the parotitis is not yet developed—a leukopenia is present. The number of lymphocytes is moderately increased and the polynuclears have fallen below normal. When the parotitis is fully developed there is a marked leukopenia, most marked in the cases with the higher temperature and greater parotid involvement. In this leukopenia the polynuclears are relatively and absolutely decreased, the mononuclears are relatively and absolutely increased.

As the disease subsides the total number of leukocytes, the polynuclears and mononuclears revert toward their normal proportion. The eosinophils, which are scarcely seen during the activity of the disease, are found in about their usual numbers as the blood returns toward the normal.

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# A CLINICAL STUDY OF HYPERTENSIVE CARDIO-VASCULAR DISEASE \*

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The study here reported was begun a year and a half ago. It was undertaken primarily with a view to determining whether or not a critical analysis of a large group of histories of persons with high blood-pressure would disclose any hitherto unsuspected relations between the symptoms or evidences obtained by objective examination and the subsequent course of the disease, on which a more exact prognosis might be based. The results of the study were reported<sup>1</sup> in part before the Section on Practice of Medicine of the American Medical Association in 1912. During the year that has elapsed since then it has been possible to obtain accurate information about a much larger proportion of this group of patients.

## I. MATERIAL STUDIED

The material studied consists of the case histories of private patients examined by my father or myself during a period of approximately nine

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\* From the Department of Practice of Medicine, College of Physicians and Surgeons, Columbia University, New York.

\* Read before the Association of American Physicians, Washington, D. C., May 8, 1913.

\* Submitted for publication, July 24, 1913.

1. Janeway, Theodore C.: A Study of the Causes of Death in 100 Patients with High Blood-Pressure, Jour. Am. Med. Assn., 1912, lix, 2106.



years, from the time when I began to make routine blood-pressure observations with the use of the wide armlet in 1903 until June, 1912. These histories are almost entirely the records of persons belonging to the well-to-do classes of society. A number have been observed personally over the whole period, but for the most part the information as to the subsequent history and the date and cause of death was obtained from the family physician originally referring the case or from his successor in charge of the patient. It is a pleasure to acknowledge the hearty cooperation received from many physicians throughout the country. Their careful answers to the inquiries made give this study much of its value.

An analysis of such a type of material has obvious defects as well as advantages. It is clear that the diagnoses of the causes of death, almost all unchecked by necropsy, have only such a degree of probability as one may expect from a group of general practitioners. This source of inaccuracy, however, was greatly minimized by the grouping which I adopted. On the question sheet sent to the physician I asked whether death had been by cardiac insufficiency, uremia, apoplexy, acute edema of the lungs, gradual anemia, pericarditis, by complicating acute infection or some unrelated disease, with a request for details. The weakness of a method of this kind is revealed by the fact that in only a single case, and that a patient seen by myself in his last illness, was pericarditis diagnosticated. For the investigation of pericarditis in patients with hypertension only a study of necropsy material is permissible. Of the other diagnoses, the majority are clinical rather than anatomical, and they may for the most part be accepted at their face value. Only in the discrimination between certain types of uremia and apoplexy is decision difficult. It is highly probable—and this was well brought out by Cabot's study of three thousand necropsies<sup>2</sup>—that apoplexy is the real cause of a certain number of deaths set down to uremia. In the consideration of my statistics, therefore, it must be assumed that the number of patients reported as dying of apoplexy is almost certainly below the true number, and that the total deaths credited to uremia contain the difference and are in excess of the facts. As in their other relations these two groups show many similarities, they can often be considered together, and the error then falls out of consideration. A small number of patients were said to have died "suddenly," without further details being given; no attempt has been made to classify these deaths further.

Questions were asked as to the existence or non-existence of certain clinical symptoms; these were an additional check on the diagnosis of the cause of death. The only statistical use which I have made of the information obtained from other physicians as to subsequent symptoms

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2. Cabot, R. C.: *Diagnostic Pitfalls Identified During a Study of 3,000 Autopsies*, Jour. Am. Med. Assn., 1912, lix, 2295.

has been in the study of the incidence of hemiplegic attacks. The errors here will all be errors of omission.

For the purpose I had in view, it seems to me that the advantages of a study of such private patients far outweigh the defects. The facts most necessary were the first symptom and the prominent early symptoms exhibited by the patient. To obtain reliable information on these points requires both an intelligent patient and a physician experienced in taking histories with reference to the particular points involved. It is evident that the conditions were fulfilled from the side of the patient. So far as the other factor enters into the situation, the vast majority of the histories were taken by myself and the remainder by my father. The earlier ones are less complete in their information on some points than those taken after I had begun to make my case records with the definite object of their subsequent scientific utilization. Here, again, undoubted errors of omission exist, and it is probable that some of the symptoms of a minor nature are less completely represented than conspicuous symptoms such as severe dyspnea, anginal attacks or edema of the lungs. Apart from this, the omissions are probably fairly evenly distributed throughout the earlier histories and will not lead to substantial inaccuracies.

The physical examinations included in these statistics were also made by my father or myself. In this way, any errors due to the personal equation are practically a constant throughout all observations, and can thus be neglected. For a satisfactory study of prognosis, only such private material can be used. A study of symptoms from hospital records is so defective as to be of little value. In the first place, ward patients are largely unintelligent and unobservant, and early symptoms are apt to pass unnoticed. In the second place, hospital historians are inexperienced and vary greatly in their ability and the care with which they interrogate the patients. In this way, a series of records running over any considerable period of years would be uneven in character, the personal equation being a wholly unknown factor. Finally, it would be practically impossible in our hospitals to obtain more than a fraction of the subsequent information which I have been able to collect about these patients. If further argument is needed in defense of the use of such clinical material lacking necropsies, for a study the aim of which is chiefly prognosis, I cannot do better than to quote the first student of the hypertension problem, Richard Bright:<sup>3</sup>

Another very important question is the length of time which this disease may exist in the constitution before it runs to its last final period: and although our experience in the hospital is great, the point of duration is yet undetermined; for, with all the advantages which an hospital affords for the multiplied accumula-

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3. Bright, Richard: Cases and Observations Illustrative of Renal Disease Accompanied with the Secretion of Albuminous Urine, *Guy's Hosp. Rep.*, 1836, i, 338.

tion of facts, there are some points on which the information derived from its wards is defective and even apt to be erroneous; and among these may be reckoned one of great importance, the probable duration of life, under any disease. If a case is much relieved, the hospital physician loses sight of it, and in all probability sees it no more; knowing nothing of future relapses or of the ultimate result. On the other hand, a very large proportion of his cases are arrived at the most advanced stage of the respective disorders: the circumstances have been such as to render them inattentive to the earlier indications of disease; and it is only when they can no longer pursue their laborious occupations, that they are driven, too late, to seek relief. Hence the physician is liable to form a wrong estimate of the progress of the disease under more favorable circumstances; and it is necessary to correct his views by a comparison with the history and results of private practice.

There has not yet, perhaps, been sufficient time, since this disease of the kidneys first attracted attention, to say to what extent life may be prolonged while the body is under its influence; but I believe, with care, its fatal effects may be kept at bay, and a hazardous life may be protracted for many years. Should that care be neglected, the chance of life will be greatly diminished.

The total number of my case records for the nine years was 7,872. Of these, 870, or 11.1 per cent., showed at some time a systolic blood-pressure of 165 mm. Hg or over. I have chosen to consider only pressures above 160 mm., because there is now substantial unanimity of opinion that, taken with the wide arm band, such pressures are always pathological. The extensive life-insurance statistics of Woley and Fisher bear this out; I have always been convinced of it.<sup>4</sup> Cook<sup>5</sup> believes that 150 mm. will eventually prove the upper limit of normal systolic arterial pressure, and I am inclined to believe him right, when readings are made with care to exclude temporary factors. When patients are seen but once, one cannot be certain of this. Therefore, it is wise to exclude from consideration all patients who have not had a systolic blood-pressure of 165 mm. or over. In addition, systolic pressures as high as 165 or 170 mm. are not infrequent in patients with severe cardiac insufficiency, in exophthalmic goiter, and at times in aortic regurgitation, and are seen transiently without a satisfactory explanation. My total group of 870 patients includes a number of instances of what seemed to me temporary hypertension, not associated with the type of disease which is the subject of this paper. These cases were eliminated from consideration, and no attempt was made to obtain information about them.

While I have diastolic readings in practically all cases, they were by different criteria at different stages of the study, and are not strictly comparable with one another. In addition, the consideration of them would so much further complicate the large amount of statistical work embodied that I have omitted the diastolic pressure entirely from consideration.

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4. Janeway, Theodore C.: *The Clinical Study of Blood-Pressure*, New York, 1904.

5. Cook: *Blood-Pressure in Prognosis*, *Med. Rec.*, New York, 1911, lxxx, 959.

Of the remaining cases, I was able to obtain definite information up to June, 1912, of 458. This group of 458 patients forms the basis of the statistical analysis which follows.

## II. SEX DISTRIBUTION

The total group of 7,872 patients contained 4,660 men and 3,212 women; the percentages of men being 59.2 and of women 40.8, or 18.4 per cent. more men than women. This preponderance of men is probably largely dependent on the fact that neither my father nor I have ever engaged in obstetrical or gynecological practice. The group of 870 patients with high pressure contains 543 men (62.4 per cent.) and 327 women (37.6 per cent.), a preponderance of 24.8 per cent. for the men.

The group of 458 patients that I have considered as having had permanent hypertension was made up of 307 (67 per cent.) men and 151 (33 per cent.) women. The preponderance of men with high blood-pressure over women is, therefore, 34 per cent., as against 18.4 per cent. for all the patients observed during the nine-year period. Subtracting the latter figure from 34 per cent., leaves 15.6 per cent. as the apparent extra incidence of hypertensive disease in the male sex. Even taking the group of 870 patients who had at any time a blood-pressure of 165 mm. or more, the male preponderance by the same circulation is 6.4 per cent.

It seems clear, therefore, that the diseases associated with persistently high arterial pressure are decidedly more common in men than in women. In all the analyses, figures for the men and women have been tabulated separately and separate curves have been drawn for the two sexes.

## III. PROPORTION OF LIVING AND DEAD

Of the 458 patients, 212 (46.3 per cent.) men and women had died by June, 1912; and 246 (53.7 per cent.) were living. Separating these by sexes, the figures are as given in Table 1.

TABLE 1.—458 PATIENTS WITH HIGH BLOOD-PRESSURE

	Whole No.	Living		Deceased		Preponderance Per Cent.
		No.	Per Cent.	No.	Per Cent.	
Men . . . . .	307	144	46.9	163	53.1	6.2 deceased
Women . . . . .	151	102	67.5	49	32.5	35.0 living
		246		216		
Difference ..			20.6		20.6	

This table brings out several striking facts: 1. The number of men who had died by the arbitrary date chosen for the completion of the statistics, June, 1912, was 6.2 per cent. in excess of the number still living. 2. For the women, the excess was 35 per cent. in the other direction; many more women were living than had died. In the same way it is seen that 20.6 per cent. more men than women had died during the same period.



It is hardly possible that accidental circumstances or differences in the distribution of men and women between earlier and later years of the nine-year period could account for so extreme a variation. I have always had the impression that the expectancy of life in women with arterial or renal disease was greater than in men. This analysis gives such impressions an objective foundation in fact. I am inclined to attribute this preponderance to the difference in mode of life, and especially in the demands made on men and women by their social environment in a group of such private patients. Women are far better able to shield themselves from influences leading to sudden rises of blood-pressure with the attendant danger of apoplexy and cardiac overstrain. In general, they can accommodate themselves more easily to the type of life best fitted to limit the progress of arterial and cardiac disease.

#### IV. AGE DISTRIBUTION

For 446 of the patients the ages were known. These ages, grouped by decades, are shown in graphic form in Chart 1.

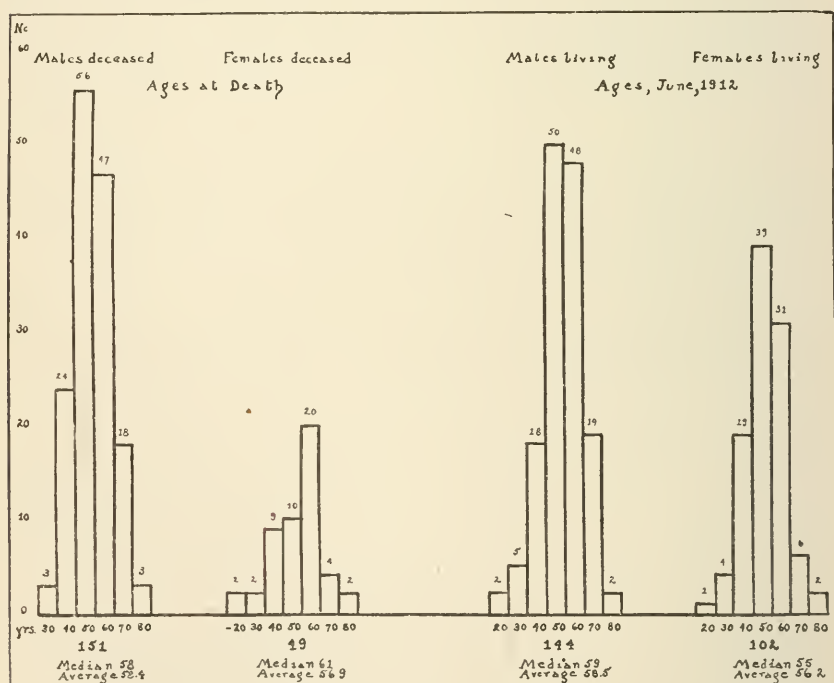


Chart 1.—Ages of 446 patients with blood-pressures of 165 mm. and over.

It is clear that the three decades from 40 to 69 years contain between 80 and 90 per cent. of all the cases. The exact proportions are given in Table 2.

TABLE 2.—AGES BETWEEN 40 AND 69 OF 446 PATIENTS, IN PERCENTAGES\*

	Males	Females
Deceased .....	84.1	79.6
Living .....	80.6	87.3

\* The percentages are computed on the basis of the totals in each case, that is, total number of deceased males, etc.

For every group, the next largest number of cases falls in the period of from 70 to 79 years. The number of patients over 80 is greater than the number below 30. This is, of course, in general conformity with all vital statistics: apoplexy, Bright's disease and heart disorders, under one of which categories these cases would be reported, show their greatest incidence above the fortieth year. On the other hand, these ages I believe are considerably higher than would be found in a similar number of patients with high blood-pressure dying in hospitals. Bright<sup>6</sup> tabulated the ages at death in seventy-four of his patients as given in Table 3.

TABLE 3.—BRIGHT'S CLASSIFICATION OF AGES AT DEATH OF 74 PATIENTS

Ages	Number
8 years.....	1
Under 30 years.....	19
From 30 to 40 years.....	16
From 40 to 50 years.....	21
From 50 to 60 years.....	13
Over 60 years.....	4
	<hr/> 24

} 51.4%

The difference between the two sets of figures is very startling: 48.6 per cent. of his patients died before reaching the fortieth year. In commenting on these cases, Bright remarks:

The youngest, whose age is given, is only 8; and there is one advanced to 73. showing, therefore, that neither youth nor age is exempt from this disease, but that it has cut off the greater part of its victims before the middle period has been attained.

Bright, of course, was dealing with cases of nephritis, acute or chronic, terminating fatally in a large London hospital. These patients did not all have hypertension, as is clear from his figures as to the proportion with hypertrophied hearts. They therefore represent some patients with the type of disease which I am considering, together with a group of the less chronic forms of nephritis. Even then, the very large proportion of persons under 40 years of age leads one to ask whether the disease from which all these young adults died has not become distinctly less common during the past seventy-five years, owing to changes in the hygiene and habits of the community. It is difficult for me to resist the impression that this is true.

6. Bright, Richard. Cases and Observations Illustrative of Renal Disease, Accompanied with the Secretion of Albuminous Urine, Guy's Hosp. Rep., 1836, i, 400.

I know of no published statistics with which mine are really comparable. Smith,<sup>7</sup> in 2,000 consecutive necropsies at the Massachusetts General Hospital, found 442 showing some arteriosclerosis. Of these, 21.3 per cent. were between 40 and 50 years of age, 29 per cent. between 50 and 60, and 25.8 per cent. between 60 and 70 — in all, 76.1 per cent. of the 442. The number of patients dying at 70 and over was double the number dying below 40 years of age. In my statistics, however, there were three times as many of the aged as of those dying before the fifth decade. The difference is mainly made up by the large number of patients in his group (19) with aneurysm, showing an average at death of 45 years, as against an average age of his cardiorenal group of nearly 55 years, of his cerebral group dying of apoplexy of 60 years, and of the patients dying of gangrene of 65 years. For the most part the aneurysm patients would not have shown hypertension during life.

I wish to call attention to the discrepancy between the average ages of the men and the women in my statistics, and the median for the ages of the two groups. There seems no question that the median is much more important than the average age from the point of view of prognosis; the median indicates a real tendency. From it, it is evident that the probability of living beyond 58 years for the men and 61 years for the women is as great as the probability of dying before that time. It also seems clear to me that, for a large proportion of these persons, the disease of which high blood-pressure was a symptom could not be looked on as materially shortening the span of life to which man has a traditional claim, but rather that it was for them an incident of growing old.

The difference of six years between the median for the women living and deceased is difficult to explain. It suggests the need for reserve in interpreting the high percentage of living women as evidence of a more favorable course of the disease in the female sex. Such ages at an arbitrary period in the course of a disease mean little in themselves. If it were possible to tabulate a sufficient series of cases terminating at or before a certain age, rather than patients living or dead at a particular date, much more definite conclusions would be warranted.

#### V. DISTRIBUTION BY HEIGHT OF SYSTOLIC BLOOD-PRESSURES

The study of the height of the blood-pressures has been the most disappointing part of this work. The blood-pressure naturally varies from day to day, from year to year. The only reliable figures that I could command were my own observations at the time of examination, sometimes one reading for a patient, sometimes many readings extending over a long period of years. I have in every case taken the highest recorded

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7. Smith, William H.: Age in Its Relations to Arteriosclerosis and Death From Arteriosclerosis, Massachusetts General Hospital Report, 1908, ii, No. 1, 185.





systolic pressure, and have arranged these pressures in groups, in different ways, in the hope that some intelligible curve of tendency might be evident. The attempt at grouping at once showed what seems to be well known to statisticians, namely, the tendency of the mind to select the nearest multiple of ten when there is a choice between it and the five below or above. This I have found to be true in surveying my blood-pressure readings. For this reason, in the final groupings shown in Charts 2, 3 and 4, the readings have been redistributed to make groups at 20 mm. intervals of pressure from 160 up. Chart 2 shows the entire number of 458 patients thus grouped according to systolic blood-pressure, with the actual readings given in the columns.

It is evident that the lower pressures predominate in the living as compared with the dead groups, the median blood-pressure for the former being about 200 mm. and for the latter 220 mm. The same tendency is shown in Charts 3 and 4, in which the blood-pressure readings in relation to the number of cases are shown in the form of curves. It is of considerable interest to note in Chart 3 of the deceased patients that as many showed a blood-pressure of 220 mm. and higher as a lower figure, both men and women. Considered from any point of view, there seems to be no tendency for lower blood-pressures among the women than among the men. In fact, a much higher proportion of women, both living and dead, showed very high readings—280 mm. and over.

A tabulation of the duration of life as it was found for 197 of the deceased after my examination of the patient, arranged by blood-pressure readings, gives the figures shown in Table 4.

The patients dying in less than a year were for the most part seen during their terminal illness and are of little value for this study. The instructive groups are those that lived for six years and over; not a single one of these patients had a blood-pressure reading below 200 mm.

It does not seem to me that any very definite prognostic conclusions can be drawn from the height of the blood-pressure. I am confirmed in this opinion by certain individual cases in which extraordinarily high pressures were tolerated for six years and more, while other patients with very moderate elevation of pressure died in a much shorter time. While, therefore, the medians of the living and the deceased groups do seem to show a certain unfavorable significance of blood-pressures above 200 mm., I consider the height of the blood-pressure a minor factor in determining the expectancy of life.

#### VI. SYMPTOMS ASSOCIATED WITH HYPERTENSION

For many years I have interrogated patients with special care in the endeavor to determine the earliest symptoms associated with cardiovascular and renal disease. These patients so frequently seek medical aid for the first time when in the advanced stages of their malady, that it

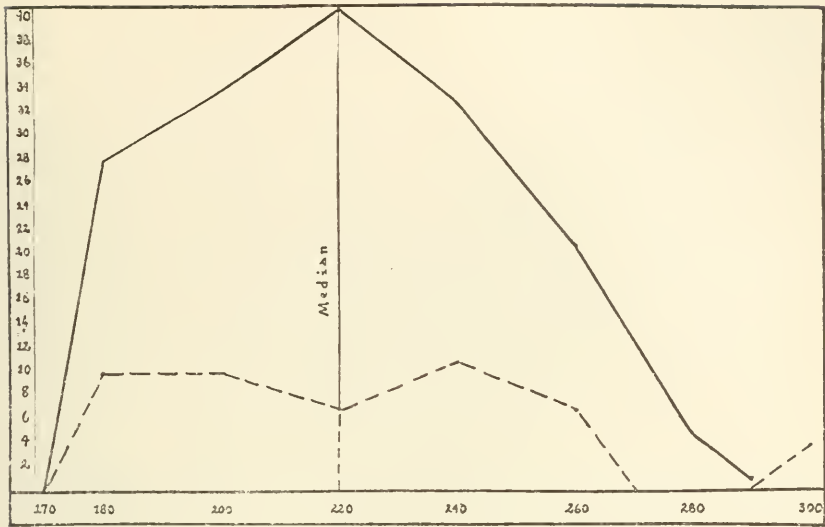


Chart 3.—Blood-pressure readings—deceased; solid line males, 163; broken line, females, 49; total, 212.

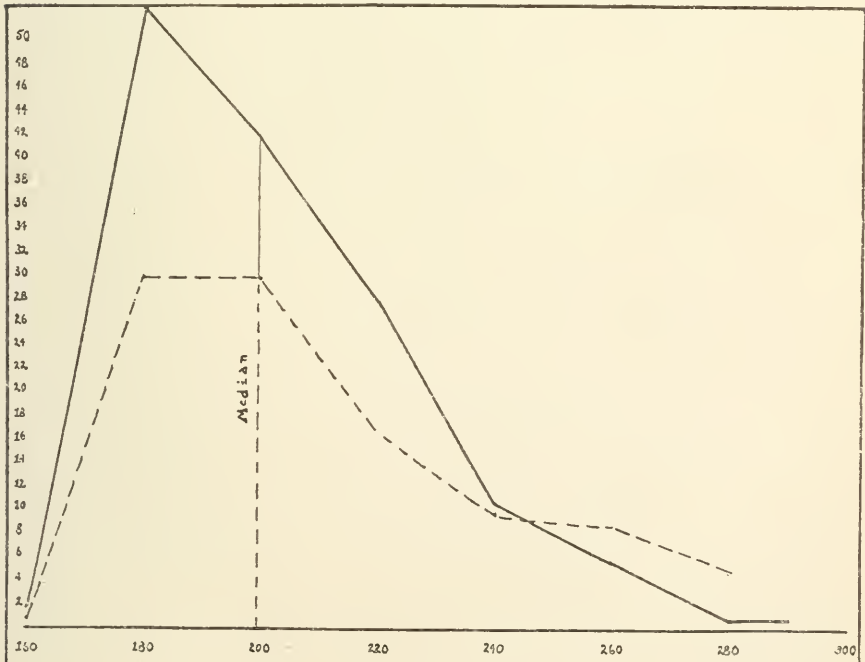


Chart 4.—Blood-pressure readings—living; solid line males, 144; broken line, females, 102; total, 246.

TABLE 4.—DURATION OF LIFE OF 197 DECEASED PATIENTS AFTER EXAMINATION AND FINDING OF HIGH BLOOD-PRESSURE

Duration	Systolic Blood-Pressure Readings in Millimeters														Totals
	165 to 170	175 to 180	185 to 190	195 to 200	205 to 210	215 to 220	225 to 230	235 to 240	245 to 250	255 to 260	265 to 270	275 to 280	285 to 290	295 to 300	
Less than 1 year.....	1	17	9	10	7	15	13	12	8	3	2	2	0	1	100
1 year.....	3	5	5	4	3	5	0	7	6	1	1	0	0	1	41
2 years.....	0	2	1	3	0	3	1	3	2	1	0	0	0	0	16
3 years.....	0	4	3	1	2	0	2	1	1	1	0	0	1	0	16
4 years.....	0	1	3	3	1	2	0	1	0	2	0	0	0	1	14
5 years.....	0	0	0	0	0	1	1	2	0	0	0	0	1	0	5
6 years and over..	0	0	0	0	1	1	0	2	0	0	0	0	0	1	5
Totals .....	4	29	21	21	14	27	17	28	17	8	3	2	2	4	197

has seemed to me of particular importance, in dealing with an intelligent group of patients, to trace back as far as possible to the first deviation from accustomed health. In many instances the task is impossible, because of preceding disease of some other nature, during which the hypertensive cardiovascular disease has developed as a sequel or concomitant. This is particularly true of diabetes in elderly subjects. With careful study, however, one may often find definite symptoms of failing cardiac reserve force, of urinary disturbance, of circulatory brain disorder, or a still less definite sense of fatigue, remembered as originating years before the physician was consulted. A knowledge of these early symptoms is so important for the recognition of the existence of serious organic disease that I feel justified in enumerating the symptoms that have appeared in this group of patients. They group themselves naturally into certain categories.

1. *Cardiac Symptoms.*—The most important numerically are the cardiac symptoms. Dyspnea stands first, beginning either with some shortness of breath on exertion or a paroxysm apparently coming out of a clear sky. The severest grade, of course, is seen in connection with the somewhat rare attacks of acute pulmonary edema. Anginoid pain on exertion has been characteristic of a considerable number of patients. Some have had purely subjective cardiac disturbances, palpitation or extrasystoles, of which they were unpleasantly aware, less often other arrhythmias. Some had paroxysmal tachycardia for years before death. Edema of the legs, while a common late symptom, frequently appeared early, and in two patients was the initial symptom.

2. *Renal Symptoms.*—Polyuria, nocturia or frequency of urination were admitted by a very large number of patients. In many cases it was not possible to determine whether nocturnal frequency indicated nocturnal polyuria or not. The elderly men with hypertrophied prostates naturally complained of nocturia, and it was not possible in them to determine from their story when polyuria was superadded. The symptom is, I believe, of great importance, especially marked degrees of polyuria. In the subsequent tabulation it has been necessary to group all those complaining of nocturnal frequency in the same category, though it is fairly certain that in a number this symptom must have been due to local causes. The significance of the symptom will, therefore, be higher than actually appears from my tabulation. Oliguria was practically impossible to determine from the patient's statement. Albumin and casts were discovered incidentally as the first evidence of disease in twelve of the deceased men and in no women. This illustrates well the advantage of life-insurance examination for the early detection of chronic disease.

The striking absence of onset with general edema of nephritic type indicates, as clearly as anything, the wide divergence of these patients



from patients with high blood-pressure as found in hospital wards. I believe, however, that were the histories of our hospital patients taken with the same degree of care, some symptoms would be found antedating the onset of general anasarca in almost all cases.

*3. Cerebral Symptoms.*—Cerebral symptoms were noted early by many patients. Headache was the most frequent. The study of significance of headache has been beset with many complications, for a surprisingly large number of these patients have been subject to migraine throughout life. Some patients have been able to name a definite date when the character of the headache changed completely; others have noted a greater severity of the paroxysms. In still others, headache has appeared for the first time with the onset of the vascular disease. So commonly have patients described to me a particular kind of headache that I have almost come to look on it as a typical nephritic or hypertensive symptom. This headache is one which appears on awakening, or wakes the patient during the early morning hours, has its greatest intensity before arising, and passes away either immediately after breakfast or during the course of the morning, to reappear in the same manner day after day for considerable periods. The intensity of the pain and its location have varied somewhat, the most severe being similar to bad migraine, and in a few cases it is attended by nausea and vomiting. The time of onset and offset has been the characteristic of greatest constancy. Throbbing has accompanied it in some cases, but not in all. I shall refer to this type hereafter as "typical" headache. Sensations of fulness or pressure in the head have been noted by many patients who did not suffer from actual headache.

The following case histories illustrate this type of headache and are of particular interest:

CASE 44.—Male, designer, aged 45. Father died of Bright's disease, mother of apoplexy. Consulted Dr. E. G. Janeway on Feb. 23, 1905, because of headaches for ten years. The headache regularly began in the morning on arising and lasted until noon. It was occipital and radiated to the vortex. During the past one and one-half years the patient had lost 35 pounds in weight. For a year he had had transient attacks of aphasia and for two months had risen once at night to urinate. He had no cardiac symptoms whatever. On physical examination he showed slight cardiac hypertrophy, extreme accentuation of the aortic second sound, moderate peripheral arteriosclerosis and a blood-pressure of 255 systolic, 170 diastolic. The reflexes were generally increased, with a slight ankle-clonus on the right. The urine was 2.160 c.c., specific gravity 1.015, with a moderate amount of albumin, and at times a few hyaline and granular casts. The patient died in October, 1906; the cause of death could not be determined.

CASE 41.—Brother of the preceding, piano-tuner, aged 37. Consulted Dr. E. G. Janeway on April 7, 1908, because of headaches. For seven or eight years he had been subject to occasional occipital headaches. For three months the headache had come daily, beginning in the occipital region and radiating forward to the frontal region. The pain was usually a dull ache, at times a sharp, cutting pain in the occiput, with no throbbing. The milder attacks began at 2 a. m., passing off toward morning; the worst ones lasted all day. The headache

awakened him out of sleep almost every night. He has had no relief from any drug except an acetanilid mixture, which he had taken in large quantity. For one year he had been getting up frequently at night to urinate and had been losing weight rapidly. Because of persistently negative urine examinations, the kidneys were exonerated from blame. On physical examination the patient showed a moderate hypertrophy of the heart, with accentuated aortic second sound, and the arteries full and tense, but without marked thickening; the blood-pressure was 230 systolic, 160 diastolic, and the pulse 100 regular. The reflexes were normal. The ophthalmoscopic examination showed slight haziness of the disk outline, fulness of the veins, and one pigmented area in the right retina, otherwise nothing. The urine was 2,200 c.c., specific gravity 1.018, without albumin but with a rare hyaline cast. On July 5, 1908, following a sea-bath the patient developed intense headache, shortly afterward became delirious and then unconscious, and died in coma at noon the next day.

CASE 77.—Male, physician, aged 39. No family tendency. Had diphtheria at 13 and severe cellulitis of arm at 28, but no other infections. Consulted Dr. T. C. Janeway on Nov. 7, 1911. Four years before, a small amount of albumin and a few casts had been found on life-insurance examination, but the patient's general health was perfect. Eighteen months before, he developed what seemed ordinary sick headache once a month. The headache would begin at about 4 a. m., becoming severe; it was generally associated with vomiting and would last until the next night's sleep brought relief. The albuminuria increased somewhat, casts became more abundant, and a few red cells were present. The headaches gradually increased in frequency and were usually relieved by vomiting. During the summer of 1911 the patient exercised hard. The headaches were no worse. For a year or two he has had to urinate once at night. For two weeks before examination there had been great polyuria (five or six times at night). He had absolutely no cardiac symptoms. He had a good appetite, but lost 10 pounds in weight. On physical examination he showed a slightly forcible apex impulse in the fifth interspace,  $\frac{1}{2}$  inches from the midsternal line, and practically normal sounds. The arteries were small and thickened. The blood-pressure was 165 systolic and 110 diastolic, and the pulse-rate 80 regular. There were normal reflexes, no edema, and normal fundus oculi. The urine (twenty hours) was 1,060 c.c., specific gravity 1.016, and contained considerable albumin, epithelium and granular casts, red blood-cells and many leukocytes. Subsequent history: On a restricted diet, occasional purgation and sweat-baths, the patient did well for a time, but had an attack of acute gout in March, 1912. By July the headaches became very severe, about five days in the week, practically always in the early morning hours. The blood-pressure rose to 210 systolic, 130 diastolic. Hypertrophy of the heart became more evident. He lost some flesh. In September, after repeated headaches, most intense in the occipital region, he developed vomiting, gradually became irrational, and the blood-pressure reached 255 mm. at the highest. He sank into a state of chronic uremia, with high blood urea, and died after forty-eight hours of coma, with rising temperature and falling blood-pressure.

Dizziness or severe attacks of vertigo have occurred with or without headache, also drowsiness and insomnia. Apoplectic attacks, varying in severity from transient aphasia to complete hemiplegia, have characterized a group of moderate size, with a definite tendency to recur in the same person, as I shall bring out later. In eight patients an apoplectic attack was the first symptom discoverable. Onset with a convulsive seizure was a great rarity. In one case, however, four years intervened between the initial uremic convulsion and death.

Failure of eyesight as an initial or even an early symptom was noted by thirteen of the deceased patients and thirteen of those still living. My

general impression of hospital and dispensary patients, without definite figures to go by, is that in them ocular disturbance is a distinctly less frequent initial symptom. This could easily be accounted for by their failure to notice the less conspicuous disturbances which an intelligent patient will remember.

4. *General Symptoms.*—Of great importance are symptoms of a general or indefinite nature, for these frequently lead to the erroneous diagnosis of neurasthenia. Unusual fatigue or exhaustion after physical exertion is the commonest. Less frequent is a sense of mental tire or of depression. Pains of various kinds, neuralgic or muscular, were the first symptoms in a number of patients. Anemia is not a common early symptom, and when progressive, has an ominous significance. I have seen it associated with anorexia, persistent diarrhea and achylia. With the onset of these symptoms, a blood-pressure usually above 220 fell to about 170 mm.

Hemorrhages of one kind or another occurred as an early symptom in nineteen of the deceased and six of the living patients. Of these patients, eight and three, respectively, had nosebleed. The other forms of hemorrhage were hemoptysis in nine patients, all deceased, and probably dependent on marked passive congestion of the lungs and gastro-intestinal hemorrhage, hematuria and uterine hemorrhage, each in a single case. Retinal hemorrhages have not been included in this enumeration, but have been grouped with the ocular manifestations. One patient came to me because he thought a subconjunctival ecchymosis indicated that he had high blood-pressure; he had had no previous warnings. Purpuric manifestations in the skin have not occurred early in any patient of this group.

Gastro-intestinal disturbances were not prominent in the early histories of these patients, and do not appear to me to bear any definite relation to the disease, apart from terminal chronic uremia. Among unusual early symptoms may be mentioned intermittent claudication or sudden giving out of the legs, or senile gangrene, all depending on arterial narrowing; also tinnitus aurium. Thirst of an intense nature I have discovered occasionally in association with polyuria, and it has been the indication of a bad outlook. Cough, in many cases depending on a pre-existing chronic bronchitis, but in some the evidence of chronic passive congestion of the lungs, has in a few cases preceded notable dyspnea.

5. *Symptoms Depending on Other Diseases.*—Diabetes has been the most common antecedent disease in this group of patients; thirty-three patients (7.2 per cent. of the whole number of 458) have been known to suffer from this for a variable length of time before the onset of any symptoms indicative of cardiovascular or renal disease. In the tabulation of polyuria as an early symptom, all cases of diabetes have been

excluded as far as possible. Prostatic or bladder disease was coincident in a number of patients. Retention of urine was the initial symptom in three of the patients, all living, and occurred very early in one of the deceased. Renal colic or the passage of a renal stone occurred in seven of the deceased and four of the living patients at an early period. Chronic bronchitis was an associated or antecedent disease in a considerable group. When severe, it necessarily introduces an error into the tabulation of dyspnea as a symptom of cardiac insufficiency associated with hypertensive arterial disease.

#### VII. CAUSES OF DEATH AND THE RELATION OF EARLY SYMPTOMS TO THEM

In my previous paper<sup>1</sup> I tabulated the causes of death and the prominent early symptoms for 100 of the cases here reported. At that time it was impossible to obtain full information for the remainder. Such information with reference to symptoms has now been secured for 212 cases, and with reference to exact causes of death for 184 patients dying prior to June, 1912. These data are shown in Table 5.

In addition, the number of times that each symptom has appeared as the first or an early symptom among the 246 patients still living on that date is shown for each vertical column. In reading the table, certain facts should be borne in mind. The tabulation is primarily one of symptoms, and several symptoms have usually occurred together in the same patient. The footings of the vertical columns, therefore, represent the number of cases in which the particular symptom has occurred before the date of my first examination, or definitely at an early period of the illness. Dyspnea on exertion and paroxysmal dyspnea occurred in association so frequently that they have been grouped in a single column, not separated as in my previous study. Only the more prominent symptoms, those occurring with considerable frequency, are here included.

The discussion of the credibility of the diagnoses under the various categories in the early part of this paper should be borne in mind. It should be especially remembered that the deaths credited to uremia presumably contain some cases of apoplexy, and that pericarditis undoubtedly occurred much more frequently. Whenever a terminal uremia carried off a patient after a long period of cardiac failure, the death has been credited to cardiac insufficiency, but apoplexy ending the scene has always been considered the immediate cause of death. Also, the endeavor has been made as much as possible to eliminate from the patients dying of edema of the lungs all those with the common, gradual terminal edema, and to include only those with acute suffocative edema of the lungs. It is not probable, however, that this has been entirely successful. The thirteen patients dying of complicating acute infectious disease comprise



Symptoms												Deaths					
Dyspnea	Edema of Lungs	Anginoid Pain	Edema of Legs	Polyuria	Visual Disturbance	Headache, Typical	Hemiplegic Attacks	Vertigo	Loss of Flesh	Fatigue, Pains	Albu., Casts, Acc. Discov.	Causes of Death	Male Pct. of Total Known, 137		Female Pct. of Total Known, 47		Total
													No.	Pct.	No.	Pct.	
48	4	11	15	11	2	3	4	2	10	7	4	Gradual cardiac insufficiency ....	48	35.0	12	25.5	60*
18	1	3	1	29	9	16	7	3	13	10	4	Uremic convulsions, coma or gradual uremia.	31	22.6	15	31.9	46†
11	0	2	3	1	2	4	5	3	3	7	1	Cerebral apoplexy or its results...	20	14.6	9	19.1	29‡
3	0	6	2	1	0	1	1	0	0	0	1	Angina pectoris ..	10	7.3	0	....	10
6	3	1	1	2	0	0	0	0	1	2	0	Edema of lungs...	6	4.4	1	2.1	7
2	0	0	0	0	0	1	1	0	0	1	0	Progressive anemia	1	0.7	2	4.3	3
0	0	0	1	0	0	0	0	0	0	0	0	Pericarditis .....	1	0.7	0	....	1
3	0	3	2	1	0	0	0	0	0	3	1	Complicating acute infectious disease	9	6.6	4	8.5	13
1	0	1	0	1	0	2	1	1	1	3	0	Other, accidental causes .....	7	5.1	2	4.3	9
11	3	7	1	5	0	3	5	0	3	3	1	Unknown .....	25	....	3	....	28
3	0	1	0	1	0	1	0	0	1	1	0	Sudden .....	4	2.9	2	4.3	6
106	11	35	26	52	13	31	24	9	32	37	12‡		162		50		212
PERCENTAGE OF TOTAL NUMBER DECEASED, 212																	
50.0	5.2	16.5	12.3	25.0	6.1	15.0	11.3	4.2	15.0	18.0	6.0						
SYMPTOMS IN LIVING PATIENTS																	
94	3	42	27	45	13	29	10	35	9	59	13§						
PERCENTAGE OF TOTAL NUMBER LIVING, 246																	
38.2	1.2	17.0	10.9	18.2	5.3	12.0	4.0	14.2	4.0	24.0	5.3						

\* These sixty cases were 32.6 per cent. of 184 known deaths. † These seventy-five cases were 40.8 per cent. of 184 known deaths.  
 ‡ All men. § Five women.

nine cases of pneumonia. "Other accidental causes" consist of automobile accidents, deaths from surgical operation, drowning, carcinoma and other wholly unrelated diseases. The six sudden deaths are presumably from angina pectoris or apoplexy.

The results of this tabulation are very similar to those obtained in the study of 100 cases already reported<sup>1</sup> (see Table 2 of that paper). They do not materially alter the conclusions then drawn. They permit, however, of more definite deductions. They may be studied from three points of view: (1) the relative proportion of deaths from the various causes enumerated; (2) the numerical relations of the various symptoms and symptom groups to one another and to the causes of death; and (3) the comparative frequency of the individual symptoms in the patients already deceased and in the group of those still living.

1. *Distribution by Causes of Death.*—The type of death was known for 184 of the 212 deceased patients; for 28 it could not be stated exactly. While the largest total number, 60 (32.6 per cent.), died a gradual cardiac death—if one groups together deaths from uremia and from apoplexy into a "cerebral" group, as did Bright, 75 (40.8 per cent.) are thus included. It is particularly interesting to note the almost exact correspondence of this with Bright's tabulation of the causes of death which he traced in 70 of his cases, 30 of the patients (42.8) dying a cerebral death with apoplexy, coma or convulsions. Cardiac insufficiency was a conception which did not exist in Bright's day, and it is impossible from his figures to obtain any clear idea of how many of his cases should be classed in this category. If in Table 5 one considers the deaths from angina pectoris, from edema of the lungs, from pericarditis, and probably some of the sudden deaths as cardiac, then it is evident that the two main groups—the cardiac and the cerebral—are almost equal, over 40 per cent. dying in each of these ways, about 12 per cent. of complicating disease, accident or unrelated causes; while a very small number (1.6 per cent.) ran the course of a progressive wasting disease with severe anemia.

A. *Sex Distribution:* At the right of Table 5 are two columns indicating the total number of men and women dying in each of the groups, and the percentage relations which these numbers bear to the total number of deceased males and females. While variation from the general sex distribution of the whole group in the three larger categories is shown only in the deaths from uremia, this difference is not so great as to be free from the possibility of chance. The next two categories, however, death by angina pectoris and by acute edema of the lungs, show a divergence from the sex distribution of the group so extreme as, I think, to indicate clearly a definite difference in sex predisposition. Not a single woman died in an anginal seizure and only one in an attack of edema of the lungs, as against, respectively, ten and six men.

*B. Age in Relation to Causes of Death:* A tabulation of the ages by decades of those dying in each group, giving the median or middle point, the mode or the largest number for any decade, as well as the second greatest number is shown in Table 6.

TABLE 6.—AGES IN RELATION TO CAUSES OF DEATH

Causes of Death	Males		Females	
	Mode*	Median	Mode	Median
Cardiac insufficiency..	50-59 60-69	50-59 ....	60-69 50-59	50-59 ....
Uremia (gradual and coma) .....	40-49 50-59	50-59 ....	60-69 40-49..	50-59 ....
Cerebral apoplexy ...	50-59 60-69	50-59 ....	60-69 ?	60-69 ....
Angina pectoris .....	60-69 50-59	60-69 ....	No women ....	.... ....
Acute infectious dis- ease (mostly pneu- monia) .....	50-59 70 and over	50-59 ....	70 and over No other	70 and over ....

\* The two sets of figures for the mode indicate the largest number and the second largest number for each group.

The number of patients dying of edema of the lungs, seven in all, is too small to use as a criterion of tendencies.

A comparison of Table 6 with Chart 1 shows that the mode, 50 to 59 in the cardiac and the apoplexy groups for the males, is the same as the mode for the total deceased males. On the other hand, for the females the mode in the cardiac, the uremic and the apoplexy groups is a decade higher, that is, 60 to 69, which agrees with the mode for the total number of deceased females as shown in Chart 1. It will be noted that the mode for the uremic group for the men (40 to 49) is a decade lower than the median for the total group of deceased; but the mode for angina pectoris (all men) is 60 to 69 years, which is higher than the average mode (50 to 59) for the men. As will be seen, the median, or the dividing point, for the men is the 50 to 59-year group for all the deaths except angina pectoris, in which it is a decade higher; while for the women, a median of 50 to 59 years applies only to the cardiac and uremic, being a decade higher for the apoplectic, and still a decade higher (70 years and over) for those dying of acute infectious diseases.

A study of the second largest groups is of interest as showing tendencies. For the men dying a cardiac death there seems to be a tendency toward a decade higher than the median for that group, and for the women there is a tendency to approach the median. In the second largest

group for the uremic men there is a tendency of coincidence with the median, while for the women there is an apparent tendency for death at an earlier age, 40 to 49 years. The tendencies for the men dying of apoplexy, as expressed in modes and median, are exactly like those of the men dying a cardiac death.

It seems probable that this table furnishes an indication of the composite nature of the uremic group, made up in part of elderly arteriosclerotics, in part of patients with contracted kidneys, and younger persons with inflammatory kidney lesions.

*C. The Relation of Blood-Pressure to Causes of Death.*—Table 7 shows from various points of view the relation between the height of the observed blood-pressure and the final cause of death.

TABLE 7.—HEIGHT OF BLOOD-PRESSURE IN RELATION TO CAUSE OF DEATH

	Special Group		Percentage at or Below Median (220) for Whole Group	Percentage Above Median (220) for Whole Group
	Mode	Median		
Cardiac insufficiency..	215-220	210	68.3	31.7
Uremia (gradual and coma) .....	215-220	220	52.1	47.9
Cerebral apoplexy.....	225-230	225	41.4	58.6
Angina pectoris.....	175-180	200	80.0	20.0
Edema of lungs.....	245-250	240	28.6	71.4
Acute infectious disease (mostly pneumonia) .....	175-180	185	84.6	15.4

NOTE.—Of the "sudden" deaths not included in the foregoing there were five in all—four men and one woman. The blood-pressures of the men were: One 175, two 210, one, 240 mm.; and the one woman had a pressure of 260.

The figures speak for themselves and require little comment. The differences are so striking as practically to preclude the operation of chance, except in the case of the small group of patients (seven in all) dying of acute edema of the lungs. Both the mode and the median for this group and the number of cases over 220 mm. are the highest noted for any of the groups. It is significant that the two cerebral groups, those dying of uremia and of apoplexy, show a tendency to marked hypertension, the apoplexy cases especially, while the patients dying of gradual cardiac insufficiency have a median of blood-pressure below the median for the entire group, and those dying of angina pectoris a still lower one, the lowest of all being found in the case of patients dying of acute infections. This table would seem to indicate that, other things being equal, a systolic blood-pressure persistently above 200 mm. constitutes a certain presumption in favor of termination by uremia or apoplexy.



The discussion of factors bearing on duration of illness, from the first discoverable symptoms to death, involves a number of considerations which must be taken up in relation to the symptoms and will, therefore, be discussed subsequently.

2. *Distribution by Symptoms.*—Table 5 shows the number of times that each of the more important symptoms has been mentioned in the anamnesis of these patients. Since the vast majority complained of more than a single symptom, it must be clearly understood that the footings of the vertical columns represent only the total number of deceased patients who had at some time during the early part of their indisposition noted the particular symptom tabulated in that column. The totals of the vertical columns must not be added together and compared with the total number of patients. The four columns to the left of the double ruling contain the symptoms referable to the cardiac changes associated with high-blood pressure. The columns to the right contain symptoms referable to the kidney and the eyes, circulatory and toxic cerebral manifestations, symptoms due to disturbed nutrition, and the indefinite fatigue or pains which are often complained of.

The most striking fact about the table is the frequency of dyspnea as compared with any other symptom. It occurred in exactly half of all the patients. The next most frequent symptom was urinary disturbance, complained of by somewhat less than a quarter of all the patients. It must be remembered that this includes cases of urinary disturbance from local causes which could not be separated from those of the patient with true nephritic polyuria. The number of patients with anginoid pain on exertion (35 in all) will, I think, seem surprisingly large to those whose experience is mainly in hospital wards; and on the other hand, the 13 patients with early disturbance of vision will seem few. Both symptoms, I believe, indicate that persistent high blood-pressure in such a group of well-to-do persons is the evidence of what is predominantly a circulatory rather than a renal disease. This is again indicated by the early occurrence of hemiplegic attacks in 24 of the patients, and of edema of the legs in 26. That 37 of the patients should have sought medical advice largely because of unusual fatigue or of neuralgic or muscular pains suggests the need of great caution in making the diagnosis of neurasthenia in persons past middle life. It should never be made without an instrumental determination of the blood-pressure. In only 12 of the 212 deceased patients was the existence of disease revealed by the discovery of albumin and casts in the urine, and in only four by the discovery of high blood-pressure in men who considered themselves in perfect health and who, on cross-examination, could not remember any departure from normal health.

Had the anamnesis not been carefully taken with reference to the existence of symptoms easily overlooked by the patient himself, the num-

ber of these accidental discoveries would probably have been greatly increased. They demonstrate the value of systematic examinations in revealing unsuspected disease and suggest the wisdom of an occasional examination of the urine and the blood-pressure in all persons past middle life. A yearly examination by the family physician of all his patients should become the rule. It is certainly as much called for among the elderly as among schoolchildren, and is in line with the progress of preventive medicine. But a careful physical examination is not the sole means of detecting disease. It is equally important that the physician should understand the art of history-taking and the significance of symptoms.

*A. The Relation of Blood-Pressure to Early Symptoms.*—The relations of the blood-pressure reading to some of the symptom groups is shown in Table 8.

TABLE 8.—HEIGHT OF BLOOD-PRESSURE IN RELATION TO PROMINENT EARLY SYMPTOMS

Symptoms	Deceased Blood-Pressures				Living Blood-Pressures			
	165-220 Per Cent.	Over 220 Per Cent.	For Special Groups		165-220 Per Cent.	Over 220 Per Cent.	For Special Groups	
			Modes	Median			Modes	Median
Dyspnea .....	56.6	43.4	235-240 185-190	220	57.4	42.6	175-180 215-220	200
Anginoid pain .....	68.6	31.4	175-180	195	78.6	21.4	175-180 185-190	190
Polyuria .....	44.2	55.8	215-220 225-230	230	75.6	24.4	185-190	200
Headache, typical....	51.6	48.4	215-220 225-230	220	72.4	27.6	215-220 195-200	210+
Hemiplegic and apo- plectic attacks .....	47.6	52.4	225-230	220+	88.9	11.1	185-190	190
Vertigo .....	44.4	55.6	215-220	220+	71.4	28.6	175-180	200
Loss of flesh.....	53.1	46.9	215-220	220	88.9	11.1	175-180	180+
Neurasthenic symp- toms (fatigue and pains) .....	62.2	37.8	185-190 175-180	215	83.1	16.9	175-180 165-170	190+
Albumin and casts (accidental discov- ery) .....	58.3	41.7	215-220	220	76.9	23.1	185-190	190+

NOTE.—Computations have been made on the basis of 220 mm., which is the median for the group of deceased patients.

The main indications which this table afford are that the patients with anginoid pain and with the indefinite neurasthenic symptoms tend to run below the median blood-pressure, while the patients with headache, polyuria and apoplectic and hemiplegic attacks tend in the opposite direction.

*B. The Relation of Early Symptoms to Causes of Death.*—A comparison of the frequency of individual symptoms in the different groups arranged by causes of death (Table 5) brings out certain prominent clinical types which hypertensive cardiovascular disease assumes. Cardiac symptoms occur three times as frequently in patients who eventually die a cardiac death as in patients who die a uremic death, and five times as often as in those who die of apoplexy. What I have previously designated as "typical" headache, on the other hand, has occurred in sixteen patients who subsequently succumbed to uremia as against three who died a cardiac death, and four who died of apoplexy. Urinary disturbance was more than twice as frequent in the group terminating by uremia, and visual disturbances more than four times, notwithstanding the fact that the total number of patients was only three-fourths of the total dying of heart-failure. The relation of hemiplegic attacks to the eventual death is less striking, but taken in relation to the totals, it indicates about a double frequency of this symptom in those dying a cerebral death of one or the other form. The relation of anginoid attacks to the probability of death in an anginal seizure is a matter of some interest. Four of the ten patients dying of angina pectoris did not have anginal attacks early in the disease, and of thirty-five patients who complained of definite precordial or substernal pain or oppression on exertion, only ten finally died in an anginal paroxysm. Eleven persons with anginoid pain died a gradual cardiac death. In the deaths from angina pectoris and acute suffocative edema of the lungs, however, previous attacks of a similar nature figure in the anamnesis of a high proportion of the patients.

*3. The Relation of Early Symptoms in the Deceased and Living Groups.*—The relative frequency of certain of the early symptoms among the deceased as compared with the living (Table 5) shows interesting resemblances and still more interesting differences. Dyspnea was a little less frequent among the living; early anginal pain and early edema of the legs were almost identical. Attacks of edema of the lungs, however, were conspicuously less numerous in the living, which rather confirms my belief in their serious prognostic significance. Urinary disturbances and typical headache show about the same relations as the dyspneas in the two groups. The accidental discovery of albumin and casts likewise occurs in the same ratio, but among the living were five women; there were no women with this finding in the deceased group. This indicates perhaps an increasing knowledge of this type of disease among the laity and consequent increase in precautionary visits to a physician. Hemiplegic attacks were much less frequent among the living. The whole question of the significance of apoplectic attacks, however, will be considered by itself later. Vertigo or dizziness, judged by its incidence in the two groups, must be considered a symptom of trivial moment. It

seems to be decidedly more common in the elderly arteriosclerotic patients than in those whom one would classify as cases of primary contracted kidney. Visual disturbances show a rather unimportant difference, which it is difficult to explain.

No symptom was more strikingly different in the group of the deceased and the living patients than loss of flesh. From this, I am inclined to the conclusion that this symptom must be looked on as of serious omen. In order to be quite safe in such a conclusion, however, I restudied the records with reference to the duration of illness in the deceased patients showing this symptom and the time before death when emaciation began. Of the thirty-two deceased patients who showed this symptom early, nineteen died in five years, thirteen in less than four years, and eleven lived six years and over from their initial symptom. In these patients, loss of flesh was manifested in three ways. One patient lost a moderate amount of weight at the start of her illness, some of which was regained subsequently. She died five and one-half years later. Six patients exhibited a slow and steady decline in weight of only moderate extent, over periods varying between five and eleven years. These two types of loss in weight are evidently without serious import. Seven patients, however, showed progressive and marked loss of flesh, three dying in less than one and one-half years, one in two and one-half years and the remaining three in from three to three and one-third years after emaciation set in. It seems to me conclusive from this that rapid, continued loss of flesh must be considered as pointing toward a fatal termination in less than the average time for patients with high blood-pressure.

Fatigue and pains, on the other hand, showed a relation to an earlier stage or a milder type of disease, by occurring almost twice as frequently in the patients still living.

#### VIII. ADDITIONAL DATA FROM HISTORIES AND PHYSICAL EXAMINATIONS

1. *Apoplectic Attacks*.—An attempt was made to discover the total number of deceased patients in whom apoplectic or hemiplegic attacks, or their analogues, occurred at any time in the course of the disease. They were mentioned by fifty-eight patients in all, forty-three men and fifteen women. These are, respectively, 21.1 per cent. of the total males and 31.2 per cent. of the total females, whose type of death is known. The sex incidence of apoplexy as a cause of death or of hemiplegic attacks during life is not materially different in the two sexes.

2. *Anginal Attacks*.—I have been especially interested in the frequency of anginoid pain among these patients. Of the deceased, 32, or 19.6 per cent. of all males, and 6 women, or 12.2 per cent. of all females, had this symptom in some degree. Among the living, however, it is



surprising to find that 20, or 19.6 per cent. of all the women, complained of anginoid pain as against 26 men, or 18 per cent. of all the males. When one compares this with the deaths from angina pectoris, all ten of which were in men, the only reasonable explanation would seem to be that the milder anginoid attacks are commoner in women than in men, and that when they occur, women avoid sudden and forced exertion and excitement far better than men; for this reason, for the most part, they escape death in an anginal paroxysm. For the rest, it is probable that syphilitic aortitis is commoner among men than among women, and is the cause of some of the anginal deaths.

3. *Cardiac Arrhythmias*.—My record of the existence of arrhythmia among these patients depends either on my detection of it at the time of examination, on the definite knowledge of it obtained from the patient's physician, or a definitely satisfactory description of the arrhythmia by the patient himself. My data are, therefore, defective, and greatly underestimate the total frequency of arrhythmia which would have been found had these patients been followed throughout a period of years. Of the 212 deceased patients, 71 had some degree of cardiac insufficiency at the time of examination. Only 8 patients had the perpetually irregular pulse indicative of auricular fibrillation. It is noteworthy to record that 6 of these 8 patients were treated with digitalis and the results were known: the result was satisfactory in four, slight in one and without benefit in the other. Contrasted with this were 6 patients with failing hearts and regular rhythm, of whom 4 responded well to digitalis, two brilliantly. The relief of dyspnea was especially notable as Miller<sup>8</sup> reported last year. Of the other arrhythmias, extrasystoles were noted in 9 patients, paroxysmal tachycardia in 3, heart-block in one, and an unspecified arrhythmia in one. Eight of the patients complained of serious palpitation. Among the 246 living patients, only 25 had insufficient hearts when examined. Only 2 patients had the evidence of auricular fibrillation; 17 patients had extrasystoles, 4 paroxysmal tachycardia, one heart-block. One a marked sinus arrhythmia and one an unspecified irregularity.

I have been somewhat impressed with the less frequent occurrence of fibrillation of the auricles in the insufficient heart secondary to high blood-pressure as contrasted with valvular disease. My evidence for this is not altogether satisfactory, as it rests in part on the incomplete figures just given. A study of the records of 75 patients with auricular fibrillation, electrocardiographically recorded by Dr. Stuart Hart at the Presbyterian Hospital, showed that 14 had a blood-pressure above 180 mm.; this is only one-fifth. This figure could have absolute value only if it were known what proportion of the cases of cardiac insufficiency at the hospital were due to hypertensive disease and what proportion to valvular disease during the same period. This I have been unable to calculate.

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8. Miller, Joseph L.: Tr. Assn. Am. Phys., 1912, xxvii, 137.

4. *Evidence of Cardiac Hypertrophy.*—My records contain statements as to the existence of evidence of cardiac hypertrophy and its degree in 193 of the deceased patients and 244 of the living. What I have considered such evidence has been enlargement of the percussion area, displacement of the apex beat downward and to the left, or a definite lifting character of the impulse, singly or combined. No roentgenographic studies have been made. The accentuated aortic second sound has not been considered such evidence, but has been tabulated separately. Table 9 has been prepared from my records.

TABLE 9.—EVIDENCE OF CARDIAC HYPERTROPHY IN 437 PATIENTS

	Deceased*	Living
None .....	8	89
Questionable .....	4	5
Slight .....	41	98
Moderate .....	96	83
Marked .....	44	19
Total .....	193	244

\* For nineteen of the deceased patients the data as to existence of non-existence of hypertrophy were not recorded.

This table, of course, does not indicate the existence or non-existence of cardiac hypertrophy, but only my ability to detect it. It is interesting especially as an indication of the very considerable number of patients in whom even a careful physical examination will fail to show a hypertrophied heart, and that only the sphygmomanometer makes the diagnosis of this type of cardiovascular disease definite. The tabulation is particularly valuable, therefore, as indicating the importance of instrumental blood-pressure observations in diagnosis. Of further interest is the difference between the two groups of patients. Not only was proof of cardiac hypertrophy lacking in nearly five times as many of the living as the dead, but the slighter grades of hypertrophy were numerically more frequent and the marked grades much less. It is altogether probable, therefore, that the group of the living is made up of persons at an earlier stage of the cardiovascular disease.

5. *Accentuation of the Aortic Second Sound.*—A definite note as to the character of the aortic second sound was made on 163 of the deceased and 226 of the living patients. My findings are shown in Table 10.

It will be seen that no accentuation of the aortic second sound could be heard in a considerable proportion of the cases — in thirty-eight (23.3 per cent. of the 163 deceased for whom this information was recorded), and sixty-seven (29.6 per cent.) of the living. This information is within

the limits of the personal equation of the examiner — absolute, not merely relative, as are the data regarding cardiac hypertrophy. The causes for absence of accentuation of the aortic second sound in hypertension are various; emphysema of the lungs, marked obesity, senile rigidity of the thorax and other unknown conditions modify the transmission of vibrations. Beyond these, it seems to me clear that there are conditions within the heart and aorta in these patients which modify the intensity and pitch of the aortic second sound in addition to the aortic blood-pressure. For instance, over the dilated aorta of syphilitic aortitis an extremely loud, ringing sound may be heard when the blood-pressure is normal. In this connection I wish to emphasize again the fact that without instrumental blood-pressure determinations, many cases of hypertension must inevitably be overlooked, even by the most expert examiner, as in many of these patients not a hypertrophied heart, an accentuated second sound, or a pulse which impresses the examiner as tense will be

TABLE 10.—INCIDENCE OF ACCENTUATION OF AORTIC SECOND SOUND IN 389 PATIENTS

	Deceased	Living
None .....	38	67
Questionable .....	1	1
Slight .....	21	55
Moderate .....	75	88
Marked .....	28	15
Total .....	163	226

found. So far as the further evidence of the table goes, very marked accentuation of the aortic second sound was proportionately more than twice as frequent in the deceased as in the living groups, and moderate accentuation was about equal, the slighter degrees greatly preponderating in the living. This shows a rough correspondence with the median for the blood-pressures of the two groups, as would be expected.

6. *Evidence of Arteriosclerosis of the Peripheral Arteries.*—The histories studied include definite statements as to the condition of the larger peripheral arteries in 171 of the deceased and 231 of the living patients. My findings are shown in Table 11.

Of particular note in this table is the large number of patients in each group in whom the radial, brachial and temporal arteries were free from evident thickening, elongation or calcification. It demonstrates how independent permanent high blood-pressure is of arteriosclerosis in the ordinary sense, that is, arteriosclerosis of the larger accessible peripheral vessels. It is the reverse side of the well-known fact that extensive

arteriosclerosis of these vessels is frequently found associated with blood-pressure not above the normal. The slight degrees of arterial thickening are especially evident among the patients still living and the marked among those that have died. The evidence here, as for cardiac hypertrophy, is therefore in the main that arteriosclerosis of the larger peripheral vessels should be looked on as a secondary lesion similar to

TABLE 11.—EVIDENCE OF ARTERIOSCLEROSIS IN 402 PATIENTS

	Deceased	Living
None .....	65	117
Questionable .....	1	0
Slight .....	14	46
Moderate .....	60	55
Marked .....	31	13
Total .....	171	231

the cardiac hypertrophy. This is further brought out by an examination of the eventual causes of death in those patients who showed moderate or marked degrees of arteriosclerosis. By "slight" arteriosclerosis in the preceding tabulation I have meant arteries which were just distinctly palpable when empty, or showed slight tortuosity. This is so regular a finding in persons past middle life that it does not seem to me to have

TABLE 12.—THE RELATION OF MODERATE OR MARKED ARTERIOSCLEROSIS TO CAUSES OF DEATH IN NINETY-ONE PATIENTS

Causes of Death	Moderate	Marked	Totals
Gradual cardiac insufficiency..	13	6	19
Uremia .....	18	13	31
Cerebral apoplexy .....	10	3	13
Angina pectoris .....	1	0	1
Edema of the lungs.....	2	3	5
Acute infectious disease.....	1	1	2
Other causes, unrelated, etc...	10	2	12
Unknown .....	5	3	8
Total .....	60	31	91

any strict relation to the disease here studied. The considerable degrees of arteriosclerosis, however, are evidence of more than mere wear and tear. A tabulation of all the cases showing more than the slighter grades is shown in Table 12.

To my great surprise, Table 12 shows a marked preponderance of disease of the large peripheral arteries among the patients dying of uremia. I had expected that the group dying of insufficient hearts would



contain the bulk of the arteriosclerotics. My general impression was that arteriosclerosis was associated with sclerotic changes in the coronaries and consecutive myocardial, nutritional disturbances, leading to myocardial insufficiency. The examination of these histories has shown, however, that these striking degrees of arterial thickening and elongation frequently occur in young persons with the picture of so-called primary contracted kidney or other primary renal disease. Some of these patients have been under 40 years of age. But again, a large number of the patients with anginoid pain and presumptive coronary sclerosis have been free from any traces of peripheral arterial thickening. This emphasizes what is beginning to be more generally appreciated: that the distribution of arteriosclerotic lesions is most variable; that extensive cerebral or coronary arterial disease may exist without any evidence of thickening of the accessible arteries of the extremities; that certain causes for arterial disease, the *Spirochaeta pallida*, for instance, have a strict elective affinity for limited portions of the arterial system — all facts tending to suggest that the arterial system cannot be looked on as a whole in the development of its pathological processes, but that local differences in susceptibility to disease exist, and that local causes play a considerable part in the distribution of its lesions.

Further, this table strengthens the argument drawn from the preceding table (Table 11) that peripheral arteriosclerosis in hypertensive disease must be looked on usually as result, not cause. This view seems to be taken by Jores,<sup>9</sup> and has been a common one among clinicians. It also fits in well with Marchand's studies in arteriosclerosis,<sup>10</sup> which go to show that cardiac hypertrophy is not as a rule associated. For this reason it seems to me that to speak of the type of disease under discussion as arteriosclerosis or hardening of the arteries, as is so frequently done, is altogether erroneous and productive of false views of the causation of high blood-pressure.

7. *Urinary Findings. A. Albumin and Casts in the Urine.*—Careful urinary examinations were made in the majority of cases at the time of my physical examination. Only the analyses made in my own laboratory or in that of my father have been considered in studying the significance of these urinary findings. The significance of this information is wholly diagnostic. I have no data from which any statement can be made as to incidence of albuminuria and cylindruria during the course of hypertensive cardiovascular disease. It was of interest from the point of view of the usefulness of a single urine analysis, as a means of diagnosis of this type of disease, to tabulate my findings.

9. Jores: Ueber die Beziehungen der Schrumpfnieren zur Herzhypertrophie vom pathologisch-anatomischen Standpunkt. Verhandl. deutsch. path. Gesellsch., 1908, xii, 187.

10. Marchand: Verhandl. d. Cong. f. inn. Med., 1904, xxi, 60.

Of the 212 deceased patients, 21 showed neither albumin nor casts in the urine when examined; and of the 246 patients still living, 95 had urines negative in both these respects. In 23 of the deceased and 19 of the living, albumin was found without casts: in only two and one case, respectively, in any considerable amount. In 17 of the deceased and 32 of the living, casts were discovered in urine which did not show albumin by the nitric acid ring test; again, in only one and two patients, respectively, were they numerous.

Of the deceased patients, 114 out of the 182 of whom I have definite information in this respect, or 62.6 per cent., had definite amounts of albumin and casts; of the living, 85 out of 236 of whom I have records, or 36 per cent., showed both albumin and casts. In 25 of the former and 42 of the latter there were but few casts and a trace of albumin. In the remainder, 89 (48.9 per cent.) of the deceased and 42 (17.8 per cent.) of the living, albuminuria was moderate or marked: and casts, except in a few cases, were numerous. No study as to types of casts has been made. Information on this point, which is beginning to come from the life-insurance companies in such studies as those of Barringer,<sup>11</sup> will have much more positive prognostic value.

A tabulation of those cases showing more than a trace of albumin with casts, in which the causes of death were known, is given in Table 13.

TABLE 13.—RELATION OF MODERATE OR MARKED ALBUMINURIA WITH CASTS TO CAUSES OF DEATH IN SEVENTY-NINE PATIENTS

Causes of Death	Number
Gradual cardiac insufficiency .....	24
Uremia .....	30
Cerebral apoplexy .....	8
Angina pectoris .....	3
Edema of the lungs .....	3
Progressive anemia .....	2
Pericarditis .....	1
Other, accidental, causes .....	5
Sudden .....	3
Total .....	79

It is clear that a negative urine examination has the very slightest value in excluding the existence of cardiorenal disease of the type presented by these patients. This, of course, is well known, but cannot too often be insisted on. These urinary studies also bring out the mixed character of the material investigated. Large amounts of albumin and large numbers of casts belong especially with the true chronic inflammatory lesions of the kidney. They are also to be expected during periods of marked cardiac insufficiency. For this reason, the deceased group showed their presence much more frequently than the living group.

11. Barringer, Theodore B.: The Prognosis of Albuminuria with or without Casts, *THE ARCHIVES INT. MED.*, 1912, ix, 657.

*B. Specific Gravity of the Urine.*—Twenty-four-hour urine specimens were not obtained from the majority of the persons studied. The urine specimens examined, however, were always mixed specimens from afternoon, night and morning, representing about sixteen hours out of the twenty-four. The records of specific gravity obtained in such specimens have only a relative value, and they are introduced here without a desire to draw more than very guarded conclusions.

TABLE 14.—SPECIFIC GRAVITY OF URINES, DECEASED AND LIVING

	Deceased	Living
Normal, 1.015 to 1.022.....	68	135
Low, below 1.015 .....	78	43
High, above 1.022 .....	25	50

TABLE 15.—SPECIFIC GRAVITY OF URINES IN RELATION TO CAUSES OF DEATH \*

Causes of Death	Urines				Total Cases
	Normal	Low	High	Unknown	
Cardiac insufficiency.	23	24	4	9	60
Uremia .....	13	24	5	4	46
Apoplexy .....	10	9	5	5	29
Angina pectoris.....	5	3	2	0	10
Edema of the lungs..	3	1	2	1	7
Acute infectious disease .....	5	1	4	3	13

\* The patients dying of progressive anemia, pericarditis, unknown, and sudden deaths are not included.

Two things stand out prominently in Tables 14 and 15: first the double frequency of low specific gravity in the whole deceased group as against the whole living group; secondly, the finding of low specific gravity more frequently than normal or high specific gravity definitely only in those patients dying of uremia. In those dying a cardiac death, about an equal number showed a low as a normal specific gravity; in those dying of apoplexy and from intercurrent infections, the greater number had urines of normal or increased specific gravity. The relation, therefore, is manifest between a low specific gravity and the type of disease in which symptoms of renal insufficiency were prominent. On the other hand, low specific gravity urine occurred with such numerical frequency in individual cases of the other types as to have only relative value in prognosis.

*8. The Relationship of Diabetes to High Blood-Pressure.*—The 458 cases studied contain thirty-six (7.9 per cent.) diabetics. This number is

probably well above what it might be with a different selection of material, for my practice has included a rather unusual number of cases of diabetes. The proportion, however, is obviously too large to be explained by mere chance. The effect of diabetes on blood-pressure has been the subject of some discussion. Potain<sup>12</sup> included diabetes with chronic nephritis as one of the causes of extreme hypertension. My view has always been that the effect is altogether indirect,<sup>13</sup> the result of the well-known frequency of arterial disease in elderly diabetics. The relation here may well be a double one—primary arterial changes in the pancreas producing the diabetes, or the diabetes leading to wide-spread arteriosclerosis. Certainly, pure diabetes in young persons, leading to death by coma, never is associated with high, but usually with low blood-pressures. My views have been substantiated by Elliott's<sup>14</sup> studies. In all but three of these patients, as already mentioned, the diabetes was recognized before the high blood-pressure or symptoms clearly referable to it were discovered.

#### IX. DATA BEARING ON DURATION OF DISEASE

In dealing with conditions of such insidious onset, in which the lesions without doubt long antedate the development of symptoms, it is manifestly impossible to arrive at an exact estimate of the possible or even usual duration. I have endeavored in each case to collate from my records the facts bearing on duration. These facts are: (1) the length of time before death during which any symptoms reasonably attributable to cardiovascular or renal disease existed, calculated to the date of death in the case of the deceased and to June 1, 1912, in the case of the living; (2) the duration of life after high blood-pressure was detected by me, or in the case of the living, the time which had elapsed between my finding of hypertension and June 1, 1912.

1. *Duration of Life from First Symptom.*—The first set of facts is shown in graphic form in Charts 5 and 6, which represent complete information on this point obtained about 187 deceased and 244 living patients. The character of the curves for the living and the deceased is strikingly similar. It is probable that a sufficiently large number of patients would obliterate the lesser irregularities. The median, also, is identical—four years for men and five years for women, in both the deceased and the living groups. The high point of the tabulation for the deceased is reached at one year, while for the living a second apex is

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12. Potain, C.: *La pression artérielle de l'homme à l'état normal et pathologique*, Paris, 1902.

13. Janeway, Theodore C.: *The Clinical Study of Blood-Pressure*, New York, 1904, p. 236.

14. Elliott, Arthur: *The Clinical Study of Blood-Pressure Variation in Diabetes and their Bearing on the Cardiac Complications*, Jour. Am. Med. Assn., 1907, xlix, 27.



found at three years. Whether this is more than accidental, I cannot tell. Patients dying in less than one year from the first development of symptoms represent to a much larger extent than the others, patients seen in their terminal illness, who were not in a position to give accurate data as to early and minor symptoms. The patients still living represent to a much larger extent those seen in office practice from whom much

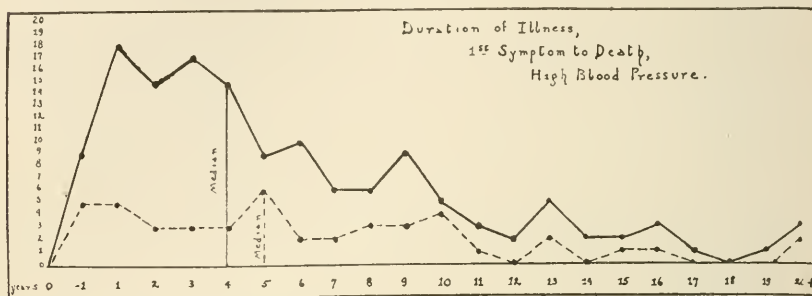


Chart 5.—Duration of illness, first symptom to death, high blood-pressure; solid line, males, 141; broken line, females, 46; total, 187.

more complete information could be obtained. It is probable that this is the real explanation of the early high point for the males in Chart 5. This much is clear, that from the time of the development of symptoms indicative of cardiovascular or renal disease, four years will witness the death of half of the men and five years of half of the women. By the

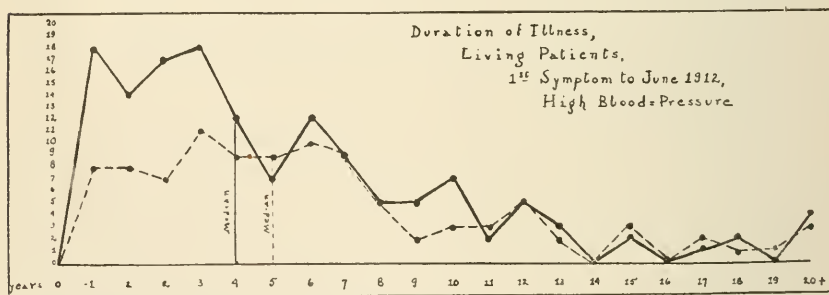


Chart 6.—Duration of illness, living patients, first symptom to June, 1912, high blood-pressure; solid line, males, 143; broken line, females, 101; total, 244.

tenth year, half of the remainder will also have died, leaving both for the men and the women a quarter of the whole group whose duration of life is ten years or over. These patients of long duration diverge from one another so widely in the length of life as to warrant entirely separate consideration; hence they have been subjected to special study. They are shown in abstract form in Table 16.

While it is impossible to discuss all the interesting features here presented, certain facts are noteworthy. Seven of the thirty-seven patients were elderly diabetics. In five of the seven, the first symptom was glycosuria or some symptom referable to the diabetes, and it was impossible to distinguish at what period of their diabetes the arterial hypertension supervened. In two, cardiac or renal symptoms occurred at the onset (Cases 103 and 182). In Case 182 the patient, a man, had general edema for fourteen years before death and anginoid pain early in his disease; he died at the age of 62, of an insufficient heart, and four years before death the blood-pressure was 170 mm. In Case 103 the patient, a woman, had edema of the legs fifteen years before her demise. She had dyspnea, both on exertion and paroxysmal, many years before death which followed at the age of 64 from apoplexy; her blood-pressure one year before death was 230 mm. systolic and 130 diastolic.

One of the most interesting patients (Case 211) was a man who at the age of 50 developed mild intermittent claudication. He subsequently suffered from neuralgic pains, vertigo and headaches, and had an attack of left-sided numbness. A blood-pressure reading taken five years before death was 185 systolic, 100 diastolic. He was drowned in the *Titanic* disaster at the age of 67, with considerable expectancy of life before him.

In three cases, 17, 86 and 172, the first symptom was accidental discovery of albumin and casts. The duration of life thereafter was 19, 22 and 13 years, respectively.

A very striking case was Case 75, in a girl who had an acute nephritis following diphtheria at the age of 9. The clinical course was characterized by a striking hemorrhagic tendency, recurring hematuria with fever, uterine and retinal hemorrhages, consecutive anemia, oliguria, with headache and vomiting. After ten years from the onset of symptoms she died of anemia consecutive to her losses of blood. The blood-pressure at the age of 19 was 210 mm. systolic and 110 diastolic. There was an associated endocarditis in her case.

In Case 178, a woman who died at the age of 78, had edema of the legs thirty years before her death, suffered from anginal attacks and paroxysms of dyspnea for eight years, had a very moderate hypertension, 170 systolic and 110 diastolic, and extensive arteriosclerosis, and finally died of lobar pneumonia.

Somewhat similar are Cases 81, 135 and 212, in men in whom the primary disease was clearly senile arteriosclerosis. In contrast to them was Case 44, in which the patient, a man dying at 46 of uremia, had his first headaches twelve years before death. He suffered from headaches, loss of flesh, transient hemiplegic attacks, dyspnea and polyuria. His blood-pressure was 255 systolic and 170 diastolic two years before death, and he died of chronic uremia. His brother (Case 41, not shown in Table 16) had an almost identical course of eight years, dying at the age

TABLE 16.—PROTOCOL OF THIRTY-SEVEN DECEASED PATIENTS LIVING TEN YEARS AND OVER FROM ONSET OF FIRST SYMPTOM

Males					
Case No.	Symptoms		Age at Death	Duration Years	Death
	First Symptom	Prominent Early Symptoms			
4	Polyuria.	Anginoid pain, polyuria, visual disturbance, loss of flesh.	70	11	Uremia.
14	Diabetes.	Gastro-intestinal hemorrhage.	63	15	Cardiac insufficiency.
17	Accidental discovery, albumin and casts.	None.	53	19	Cardiac insufficiency.
19	Anginoid pain.	Anginoid pain.	58	11	Angina pectoris.
26	Palpitation.	Paroxysmal dyspnea, albumin and casts.	52	13	Unknown.
44	Headache, typical.	Headache, typical, loss of flesh, hemiplegic attack, vertigo, polyuria.	46	12	Uremia.
48	Dyspnea on exertion.	Retention of urine.	70	16	Unknown.
67	Anginoid pain.	Anginoid pain, dyspnea on exertion, paroxysmal dyspnea, edema of legs.	58	13	Cardiac insufficiency.
81	Fatigue.	Dyspnea on exertion, slight.	72	10	Apoplexy.
86	Accidental discovery, albumin and casts.	Anginoid pain +.	52	22	Angina pectoris.
97	Renal colic.	Mental fatigue, headache typical, palpitation, neuralgic pain.	54	16	Sudden.
109	Polyuria.	None.	74	13	Pneumonia.
121	Palpitation.	Paroxysmal tachycardia.	62	32	Auto accident.
129	Albumin, casts, etc.	Albumin, casts, legs giving out, vertigo, loss of flesh.	61	12	Apoplexy.
135	Oliguria.	Dyspnea on exertion, slight.	80	15	Cardiac insufficiency.
164	Neuralgic pains.	Diabetes.	63	10	Pneumonia.
165	Diabetes.	Anginoid pain.	60	10	Angina pectoris.
172	Accidental discovery, albumin and casts.	Neuralgic pains, albumin and casts.	40	13	Cardiac insufficiency.
175	Diabetes.	Diabetes, dyspnea on exertion.	73	29	Pneumonia.
176	Palpitation.	Palpitation, vertigo, dyspnea on exertion, slight.	59	16	Cardiac insufficiency.
182	Diabetes, edema of nephritic type, Anginoid pain.	Anginoid pain.	62	14	Cardiac insufficiency.
183	Albumin and casts, paroxysmal dyspnea.	Albumin and casts, paroxysmal dyspnea, hemoptysis, dyspnea on exertion, loss of flesh, cough.	65	14	Cardiac insufficiency.

TABLE 16.—CONTINUED  
(MALES)

Case No.	Symptoms		Age at Death	Duration Years	Death
	First Symptom	Prominent Early Symptoms			
197	Paroxysmal dyspnea.	Paroxysmal dyspnea, dyspnea on exertion, polyuria.	68	10	Cardiac insufficiency.
211	Mild arteriosclerosis.	Cough, vertigo, headache typical, hemiplegic attack.	67	17	Drowned.
212	Palpitation.	Palpitation, dyspnea on exertion.	72	13	Apoplexy.
213	Diabetes.	Edema of legs, anginoid pain.	59	11	Pneumonia.
217	Edema of legs.	Paroxysmal tachycardia, pericardial effusion.	59	10	Pericardial effusion.

Females

25	Albumin and casts (pregnancy).	Loss of flesh, fatigue.	47	23	Uremia.
53	Diabetes, renal colic.	Loss of flesh, albumin and casts.	61	13	Unknown.
63	Diabetes, albumin and casts.	Neuralgic pains, albumin and casts, dyspnea on exertion, paroxysmal dyspnea, visual disturbance.	62	10	Uremia.
65	Dyspnea on exertion.	Dyspnea on exertion, polyuria, edema of lungs, palpitation.	56	10	Cardiac insufficiency.
75	Hematuria.	Hematuria, oliguria, headache typical, gastro-intestinal hemorrhage marked, gangrene of extremity, anemia.	19	10	Progressive anemia.
96	Marked gastro-intestinal disturbance, drowsiness.	Polyuria, albumin and casts.	44	13	Uremia.
103	Edema of legs.	Dyspnea on exertion, paroxysmal dyspnea.	64	15	Apoplexy.
148	Goiter.	Goiter.	55	16	Cardiac insufficiency.
178	Edema of legs.	Anginoid pain, paroxysmal dyspnea.	78	30	Pneumonia.
187	True psychosis.	True psychosis, albumin and casts, general edema, uremia.	60	11	Apoplexy.



of 37, also of chronic uremia. In these two men, headache of the kind I have described, of the most intense character, was wholly unrelieved by any methods of treatment.

Cases 19 and 67 are noteworthy as illustrating the occasional long tolerance of anginoid pain. In each of these men, this was the first and throughout the prominent symptom, yet the former lived eleven years, dying finally of an anginal seizure, and the latter succumbed to gradual cardiac insufficiency only after thirteen years.

A. *Duration of Illness in Relation to Causes of Death.*—The duration of illness from the first symptom to death is shown arranged by causes of death in Table 17.

TABLE 17.—THE RELATION OF DURATION OF ILLNESS (FIRST SYMPTOM TO DEATH) TO CAUSES OF DEATH

Causes of Death,	Duration (years)									Total	Median Year
	—1	1	2	3	4	5	6*	Per- cent- ages	Unknown		
Cardiac insufficiency .....	9	3	10	5	5	4	19	34.5	5	60	4
Uremia .....	3	8	6	10	4	4	11	23.9	0	46	3
Apoplexy .....	2	1	4	4	4	6	7	25.0	1	29	4
Angina pectoris .....	0	1	0	2	3	1	3	30.0	0	10	4
Edema of the lungs.....	0	0	3	1	1	2	0	0.0	0	7	3
Acute infectious disease...	2	1	0	2	1	0	6	50.0	1	13	5
Progressive anemia .....	0	0	0	1	0	0	2	66.6	0	3	..
Pericarditis .....	0	0	0	0	0	0	1	100.0	0	1	..
Accidental causes .....	0	1	1	2	1	1	3	33.3	0	9	..
Sudden .....	0	1	1	0	2	1	1	16.6	0	6	4
										186	

\* And over.

NOTE.—The percentages of the column, 6 years and over, have been compiled on the basis of the cases in which the death was known, eliminating the unknown.

The differences here are not very great between the groups. But, judged either by the median for duration or the number of patients living six years and over, the group dying with uremic symptoms seems to be somewhat shorter-lived; 75 per cent. of these patients died before the sixth year. There were too few persons dying of edema of the lungs to make it safe to draw any conclusions, but it is interesting that the whole seven died before the sixth year of illness. The distinctly greater duration in the case of patients dying of acute infectious diseases is of still more significance when one considers that such intercurrent infection must be looked on as an accident terminating the underlying chronic disease before it would in itself have led to a fatal issue. It seems fair to conclude, then, that these patients exhibited a less progressive type of cardiovascular disease than the others. I think it is also of some interest to observe that the duration of life in patients dying of angina pectoris

was not materially different from patients dying of gradual cardiac insufficiency; in each case, at least 30 per cent. lived for six years and over from the onset of the first symptom.

In the case of the patients living June, 1912, Tables 18, 19 and 20, of first and early symptoms, show their general character.

TABLE 18.—EARLY SYMPTOMS OF 51 PATIENTS (28 MALES AND 23 FEMALES) LIVING JUNE 1, 1912. MORE THAN TEN YEARS FROM ONSET OF FIRST SYMPTOM

Symptoms	Total No. of Patients	As a First Symptom in
Dyspnea (on exertion and paroxysmal) . . . .	20	6
Anginoid pain . . . . .	16	6
Edema of lungs . . . . .	2	0
Palpitation . . . . .	13	8
Edema of the legs . . . . .	8	1
General edema . . . . .	1	1
Polyuria . . . . .	6	2
Retention of urine . . . . .	2	2
Headache* . . . . .	12	1
Vertigo . . . . .	9	3
Hemiplegic attacks . . . . .	5	1
Transient coma . . . . .	1	1
Uremic convulsion . . . . .	1	0
Drowsiness . . . . .	1	0
Visual disturbances . . . . .	1	0
Pains (mainly neuralgia) . . . . .	8	3
Fatigue (physical and mental) . . . . .	6	1
Hemorrhages . . . . .	3	0
Loss of flesh . . . . .	2	0
Gangrene of extremities . . . . .	1	1
Intermittent claudication . . . . .	4	1
Cough . . . . .	1	0
Diabetes . . . . .	10	6
Albumin and casts (accidental discovery) .	3	3
Cardiac disturbance (accidental discovery) .	2	2
High blood-pressure (accidental discovery) .	1	1

\* Of these cases of headache, three were typical, nine atypical; the case in which headache was the first symptom was of the atypical type.

TABLE 19.—AGES OF FIFTY-ONE LIVING PATIENTS (28 MALES AND 23 FEMALES) WITH SYMPTOMS MORE THAN TEN YEARS FROM ONSET

From 30 to 39 years . . . . .	3	From 70 to 79 years . . . . .	9
From 40 to 49 years . . . . .	4	Eighty and over . . . . .	3
From 50 to 59 years . . . . .	15		—
From 60 to 69 years . . . . .	17	Total . . . . .	51

TABLE 20.—DURATION OF ILLNESS, FIRST SYMPTOM TO JUNE 1, 1912, OF FIFTY-ONE PATIENTS LIVING FOR TEN YEARS AND LONGER FROM ONSET OF FIRST SYMPTOM

Years	Number	Years	Number
10 . . . . .	12	14 . . . . .	0
11 . . . . .	6	15 . . . . .	5
12 . . . . .	9	Over 15 . . . . .	14
13 . . . . .	5		—
			51

It is evident that in a considerable number of instances, the first symptom was one of a minor nature — palpitation, vertigo, or pains, or a preceding disease, such as diabetes. There were, however, six patients who had dyspnea, six who had anginoid pain, one who had edema of the legs, one with general anasarca, one a hemiplegic attack, and one an attack of coma more than ten years ago who were still living in June, 1912. In six the discovery of their condition was purely accidental. The most significant point in the tabulation of these blood-pressures (Table 21) is that the median for this group of long duration is exactly the median for the entire living group, that is, 200 mm. This, again,

TABLE 21.—BLOOD-PRESSURES OF FIFTY-ONE PATIENTS LIVING MORE THAN TEN YEARS FROM ONSET OF FIRST SYMPTOM

Blood- Pressure, mm.	No.of Patients	Blood- Pressure, mm.	No.of Patients
165-170 .....	4	235-240 .....	3
175-180 .....	5	245-250 .....	3
185-190 .....	10	255-260 .....	1
195-200 .....	6*	265-270 .....	2
205-210 .....	8	275-280 .....	1
215-220 .....	7		
225-230 .....	1	Total .....	51

\*Median.

TABLE 22.—DURATION OF LIFE AFTER FINDING HIGH BLOOD-PRESSURE, 438 PATIENTS

Deceased (to Death)					Living (to June, 1912)			
Dying in	Males		Females		Males		Females	
	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.
Less than one year...	73	49.0	27	55.1	51	37.0	38	37.3
One year and over...	32	21.5	10	20.4	13	9.4	12	11.8
Two years and over..	14	9.4	2	4.1	13	9.4	10	9.8
Three years and over.	12	8.0	4	8.2	18	13.1	8	7.8
Four years and over..	10	6.7	4	8.2	19	13.8	12	11.8
Five years and over...	4	2.7	1	2.0	10	7.2	9	8.8
Six years and over...	4	2.7	1	2.0	14	10.1	13	12.7
Total .....	149	100.0	49	100.0	138	100.0	102	100.0

indicates that the exact height of the blood-pressure has very little prognostic value. Of course, it must be remembered that these blood-pressure readings are not in all cases the pressures of ten years ago. The study of these patients suggests the need for extreme reserve in making any prognosis as to the probable duration of life in this type of chronic disease.

2. *The Duration of Life after Finding High Blood-Pressure.*—The duration of life after my discovery of high blood-pressure in the deceased patients and in those still living is shown in Table 22.

Since my accurate readings date back but ten years, these figures are useful only as suggesting future possibilities. All but 2.7 per cent. of the men and 2 per cent. of the women died within five years of my discovery of hypertension. The few living from six to ten years later, however, show that the duration of hypertensive cardiovascular disease may easily exceed ten years. Absolute information on this point will eventually be afforded by the large life-insurance companies. An excellent start has been made in this direction by such work as that of Fisher.<sup>15</sup>

#### X. ETIOLOGY

When this work was undertaken I had hoped that it might also be possible to correlate information of value as to possible etiological factors contributing to the development of arterial hypertension. The range of such factors as were found in the life histories of these persons was so great as to demand an extensive critical study to yield anything more than the usual text-book catalogue of all the diseases and vices of the human race as the causes of any disease the origin of which is obscure. This attempt was, therefore, for the time being abandoned, in order to devote more attention to the foregoing facts bearing on the clinical features and the prognoses of the morbid state, which, while less important than a knowledge of its cause, seems also less likely to mislead. A similar discussion of the etiology seemed likely to provoke the criticism made by Artemus Ward that "it is better not to know so many things than to know so many things that ain't so."

#### XI. GENERAL CONSIDERATIONS

The question may well be asked whether there is any warrant for bringing together, as I have done, cases of so diverse a nature, which had in common only the single symptom, high blood-pressure. Throughout the preceding pages, I have purposely refrained from indicating the clinical diagnoses in these cases. I have done this because the clinical diagnoses which I made ten years ago are not the clinical diagnoses which I would make in the same patients to-day, and I trust that they are not the diagnoses under which such cases will be tabulated ten or twenty years hence. I have endeavored to limit myself to a statement of facts and obvious numerical relationships. Neither the clinical studies of nearly a century, nor the experimental investigations of nearly half a century have succeeded in elucidating the real cause of high blood-pressure, nor have the anatomical researches of pathologists resulted in any unanimity of opinion about the relationship of the underlying lesions to one another and to the disease picture. One physician would make the

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15. Fisher: *The Diagnostic Value of the Use of the Sphygmomanometer in Examinations for Life-Insurance*, Meeting of Association of Life Insurance Medical Directors, Oct. 4, 1911, New York.



diagnosis of chronic interstitial nephritis in the bulk of these patients, another of arteriosclerosis, a third would take the purely symptomatic point of view and speak of many of them as cases of arterial hypertension. But the latter cannot be a finally satisfactory concept. The adrenalinemia theory, which has had so much vogue of late, is, as I have endeavored to show elsewhere,<sup>16</sup> without foundation as yet. It is evident that a large proportion of these patients show at necropsy one or another type of kidney disease, most commonly either the so-called primary contracted kidney, or arteriosclerotic atrophy. In some, however — and I have recently had brilliant examples of this — the kidney lesions are of the most insignificant character, but wide-spread arteriolar disease exists elsewhere. The tendency is strong for pathologists to return to the original view of Gull and Sutton<sup>17</sup> that this type of disease is primarily a disease of the small blood-vessels.

The character of the lesions has been well described by Jores.<sup>18</sup> The question remains open, however, whether the high blood-pressure precedes or causes the lesion. That the arterial narrowing will heighten a pre-existing high blood-pressure and render it more permanent does not make it clear that the arterial lesion comes first. That arteriosclerosis of the larger arteries may at times extend toward the periphery and produce a similar type of disease is also clear. Beyond that, it seems probable that the high blood-pressure seen as a symptom in the more acute inflammatory affections of the kidney must be dependent on other causes than this particular arteriolar disease.

The foregoing study seems to me to show clearly, if it shows anything, that the symptom pictures presented may consist of almost any set of combinations in any of these types of disease, and that clinically a satisfactory anatomical diagnosis is almost impossible. Further study by tests of renal function may help in differentiation, but for the present the physician who goes to the necropsy table expecting to be right as to the gross or minute anatomical lesions in these patients with high blood-pressure will frequently meet disappointment. For this reason — because it emphasizes both the existence of real anatomical lesions and the relation to them of the unifying physiological disturbance — I prefer for the present to speak of all these patients as cases of hypertensive cardiovascular disease. When this condition becomes engrafted on an obvious primary inflammatory nephritis, I consider it secondary hypertensive cardiovascular disease. When it originates insidiously and the urinary

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16. Janeway, Theodore C.: Nephritic Hypertension: Clinical and Experimental Studies, *Am. Jour. Med. Sc.*, 1913, cxlv, 625.

17. Gull and Sutton: The Pathology of the Morbid State Commonly Called Chronic Bright's Disease with Contracted Kidney, *Med.-Chi. Tr.*, 1872, lv, 273.

18. Jores: *Kritisches zur Lehre von der Nephritis und den Nephropathien*, *Med. Klin.*, 1913, ix, 18.

changes are part and parcel of the picture from the start, or follow later, I consider it primary hypertensive cardiovascular disease. The results of these processes entail danger from the point of view of the heart, the arteries, especially the cerebral arteries, or of eventual kidney atrophy below the factor of safety for the human kidney.

## XII. CONCLUSIONS

1. The most prominent symptoms associated with high blood-pressure are circulatory rather than renal. The disease underlying high arterial pressure is predominantly a disease of the circulatory system, and is best designated hypertensive cardiovascular disease, either primary, or secondary when preceded by an inflammatory nephritis.

2. Death in this type of cardiovascular disease, among patients in private practice, occurs in the following ways, arranged in the order of their frequency: First, by gradual cardiac insufficiency; second, with uremic symptoms; third, by apoplexy; fourth, from some complicating acute infection; fifth, in an attack of angina pectoris; sixth, from purely accidental and unrelated causes; seventh, in a paroxysm of acute edema of the lungs; eighth, after the manner of cachexia.

3. The early symptoms associated with hypertensive cardiovascular disease have an important prognostic significance which can be utilized therapeutically, particularly for the institution of safeguarding treatment.

4. The early occurrence of symptoms of myocardial weakness, especially dyspnea, indicates a more than 50 per cent. probability of an eventual death by cardiac insufficiency. In these patients, to safeguard the heart is the main therapeutic indication.

5. The early occurrence of anginoid pain on exertion does not indicate a probability of death in an anginal paroxysm for more than one-third of the patients. It does indicate a probable cardiac death of some type. The therapeutic indications here are similar to the foregoing, except as modified by the existence of syphilitic aortitis. Anginal attacks as compared with other cardiac symptoms do not materially affect the expectancy of life.

6. Polyuria, particularly if nocturnal, indicates the probability of a uremic death for more than 50 per cent. of the patients. It is not essential to safeguard the heart in these patients, unless associated cardiac symptoms exist.

7. Headache, especially that heretofore described as typical, indicates the probability of a uremic death for more than 50 per cent. of the patients, and of the death from apoplexy for a considerable number of the remainder. The therapeutic indications are similar to those of polyuria.

8. Loss of flesh, if marked and progressive, is a symptom of bad prognostic import.

9. The relation of the height of the blood-pressure to prognosis is doubtful. Systolic pressures persistently well above 200 mm. Hg seem to indicate a greater probability of death by uremia or apoplexy. The exact height of the blood-pressure does not seem to have much bearing on the expectancy of life.

10. The average duration of life in this group of patients, after the onset of symptoms associated with high blood-pressure, has been four years for the men and five for the women. One-half of the whole number of deceased died during the first five years. One-quarter of the number lived between five and ten years, and the remaining quarter over ten years from the appearance of the first symptom. The existence of this considerable number of patients living for a long period of time suggests the need of great caution in making a prognosis as to expectancy of life.

I wish to express my indebtedness to my secretary, Miss Anna L. von der Osten, for the large amount of statistical work extending over a year and a half represented by these studies; and to acknowledge with thanks the occasional helpful suggestions on statistical problems afforded by Dr. Leonard P. Ayres of the Russell Sage Foundation.

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## BOOK REVIEWS

STAMMERING AND COGNATE DEFECTS OF SPEECH. By C. S. Bluemel. Cloth. Price, \$5 net. Two Volumes. Total pp. 756. New York: G. E. Stechert & Co., 1913.

This book treats the subject in a most original manner. The author leads up logically to his conclusions by discussing in the first chapters of the first volume many psychological facts, and especially those which pertain to speech. Different aspects of their bearing on the different phases and peculiarities of stammering are taken up. After an exhaustive consideration of the psychological conditions which are present in stammering Bluemel comes to the conclusion that "the primary cause of stammering is auditory amnesia. The secondary or auxiliary causes are bewilderment, perversion of the verbal image, autosuggestion giving rise to inhibition of the will and finally to fear."

The efforts of most systems leading to the correction of stammering have been directed toward these secondary or auxiliary causes and have been successful only inasmuch as these causes overrule the picture. For instance, the elocutionary systems will usually overcome the physical errors such as respiration, articulation, etc. They are good as far as they go, but they do not go far enough. The time beating system is, according to Bluemel, directly pernicious since it distorts the stammerer's verbal imagery. He believes that the stammerer should be encouraged to commence articulation because in this way he stimulates the action of the auditory speech center. This may be done in other ways, as, for instance, by associating certain vowel sounds with colors, or when a vowel is to be pronounced appealing to the visual rather than to the auditory sensation. He believes that the attention of the stammerer should be centered on the refractory word. He states that "if a complete and permanent cure is to be effected the stammerer must cease to depend on auditory images, for so long as he relies on these speech cues he must inevitably stammer when amnesia occurs."

In these many ways he points out the direction toward which treatment of stammering should go.

The second volume takes up the various systems now in vogue for the treatment of stammering and discusses their bearing on the subject of auditory amnesia.

These two volumes constitute a most original presentation of the subject, and certainly bear the stamp of deep and independent thought. Whether one agrees with the ideas expressed or not, he must feel that the work is a valuable contribution on the subject.

STUDIES CONCERNING GLYCOSURIA AND DIABETES. By Frederick M. Allen, A.B., M.D. Cloth. Price, \$9. Pp. 1179. Boston: W. M. Leonard, 1913.

The avowed purpose of this work is an application of such knowledge as can be gained by experiments on animals to the evolution of an improved therapy for diabetes in man. Its preparation has occupied the author three years and these must indeed have been years of most arduous toil and industry for, in bulk alone, the volume before us far exceeds anything that has ever been written in the difficult and confusing field of glycosuria and diabetes. The knowledge that so large a volume has occupied so short a time in its compilation, would ordinarily lead us to expect a considerable amount of slipshod and undigested matter, but this is by no means the case; from cover to cover the pages of the book are filled with material that is directly or indirectly applicable in such a detailed study as the work professes to be.



In general, the book consists of a literary review of previous work and of a detailed account of the author's own experiments. Since such an account has not previously been published much space is occupied in doing so, and this in part accounts for the large size of the volume.

In the first five chapters the general effects of excessive sugar assimilation are discussed. After briefly reviewing the development of knowledge regarding the nature and amount of sugar in the blood and urine the author proceeds to show how contradictory and confusing are our present-day views regarding the assimilation limits of the various sugars. He points out to what degree differences in species and in the physiological state of the animal, as well as differences in the chemical nature and method of administration of the sugar have been found to influence the results. He states that "if it is desired to rule out every possible irregularity of absorption and to test the simple saturation limit the intravenous method may be useful" whereas, "for a test of the rate at which the tissues utilize sugar the subcutaneous is the method of choice."

As an outcome of this review and supported by experiments of his own, the author concludes that a fundamental distinction exists in the power with which the organism uses sugar in normal as compared with diabetic animals. It is only in the diabetic organism that any real limit of assimilation exists, the traces of sugar which may escape in the urine of normal animals being more or less accidental and in no way indicating that an assimilation limit has been reached. This behavior of dextrose in normal animals is designated as dextrose paradox.

The disappearance of this "paradox" is the most reliable test for the existence of the diabetic state in that "it furnishes a demonstrable and absolute theoretical distinction between diabetes and every other form of glycosuria." It is pointed out, however, that it is probably only in laboratory experiments that the test is of value; in clinical practice, most cases of diabetes retain so extensive a sugar burning power that the paradox is still evident.

Several other facts of practical importance concerning the effects of excessive sugar ingestion are considered. It was found, for example, that repeated sugar injections in cats did not lower the tolerance nor did they necessarily lead to fat deposition; in fasting animals they assist the general strength and well-being, "they furnish spending money, not reserve capital;" they may therefore be useful in weakened conditions. After confirming these observations by experiments on puppies a valuable résumé is given, at the end of Chapter V, of the therapeutic use of dextrose injections.

Perhaps the most original contribution in the book concerns the effects which are produced on the excretion of urine by injections of sugar in normal as compared with diabetic animals. In normal animals of various species all the freely utilizable sugars cause anuria when given otherwise than intravenously, but by this method they all cause diuresis. In diabetic animals on the other hand, dextrose causes diuresis in whatever way it is given. Most of the fallacies which might be thought of as entering into the experiments from which these conclusions are drawn are critically considered and the criticisms appear to be adequately met. It is unfortunate, however, that more care was not taken in making certain that the bladder was emptied at the end of each observation period. The anuria and subsequent polyuria following subcutaneous injections of such irritant solutions as 80 per cent. dextrose may have been due solely to reflex bladder retention. The amboceptor hypothesis which is invoked to explain these results is at least ingenious. By subjecting the various forms of experimental glycosuria to the above diuresis-test it was found that none is truly a diabetes except that supervening on complete depancreatization.

Most interesting and well-arranged reviews are given on such subjects as the classification of the various forms of glycosuria, on adrenalin, phloridzin and the nervous system of glycosuria, on the anatomical changes in the pancreas, on therapy, etc. The chapter on the polyglandular doctrine is the best that we have read on this modern-day phantasy.

As a well written review of a great part of the enormous literature that has accumulated in the domain of experimental diabetes and as a most suggestive thesis, the book is to be highly commended. The reviews of the work of previous investigators are clearly and accurately stated and they are well arranged in relationship to one another. A most valuable bibliography completes the volume.

**IRRITABILITY.** By Max Verworn, M.D., Ph.D., Prof. at Bonn Physiological Institute. Memorial Lectures under the Stillman Foundations at Yale University. Yale University Press.

No single condition in a process in the least complex can be considered causal; rather the evolution of a process depends on all conditions being in proper order. All changes in vital conditions act as stimuli. In the present work, however, only changes in the external world are considered as stimuli: changes within the organism are considered as development.

Stimuli are considered as to their amount, quality (negative or positive), intensity, form and rapidity. Under intensity Fechner's law (the intensity of sensation varies with the logarithm of the intensity of the stimulus), and the "all or none law" (any response is the most complete response possible, i. e., the heart beat) are discussed. Stimulation also causes a qualitative change in vital processes metabolically, a practical differentiation from the constant change going on in the metabolism of rest or equilibrium of metabolism. In equilibrium of metabolism  $\frac{\text{assimilation}}{\text{dissimilation}} = 1$ , i. e., biotonus. Stimuli are likened to catalyzers: not appearing in the resultant state. They act on the "specific energy" of vital processes (eyes give only visual sensation, organs of taste only gustatory), either increasing (exciting) or decreasing (depressing) their activity. Stimuli also act to alter processes qualitatively, directing chemical changes into new channels. Examples of this are the changes occurring under constantly recurring weak stimuli as in chronic disease or constant ingestion of alcohol. Disease is life under altered conditions, and altered vital conditions are stimuli: disease brings about new conditions.

Metabolic processes must be considered as being morphologically associated in a branching arrangement somewhat after the formation of Ehrlich's side-chain diagrams with relatively stabile and labile elements. The end products of the labile elements, waste products, may act as stimuli (altered vital conditions) and cause a secondary reaction such as fatigue. Rest and restoration then come about as evidence of a self-regulatory metabolic mechanism. Development is one aspect of the constancy of metabolic activity; death is another. In the latter the stimulation of waste products is greater than metabolic restoration.

To understand the result of stimulations one must understand the latent period and the production of energy. The latent period is occupied by changes rising from zero to a point appreciable by our indicators. With the fewest possible exceptions these are at first dissimilative or breaking down changes. They affect the functional, N-free, labile elements of metabolism, and result in the easily diffusible end-products,  $H_2O$  and  $CO_2$ . Weak acids cause increased blood supply. Therefore functional hypertrophy, with increased formation of the eliminative substances, among them carbonic acid, results in increased blood supply. Increased blood supply leads to increased oxidation, more energy, and true hypertrophy. The conduction of stimuli in pseudopodia of diflagella, poorly conducting protoplasm, is influenced by intensity and distance. Apparently this is not so in medulated nerves where the all or none law rules.

As a vital process occurs (the contraction of a muscle) oxidizable substances are broken down. The destruction of these substances as well as the presence of the resultant waste products reduces irritability. These labile substances, and with them irritability, are restored by metabolic self-regulation. Where the all or none law does not hold, disintegration is not complete, sufficient stimulus can cause a reaction, and the refractory period is relative. Where the all or none law rules, there is disintegration of all labile substances, irritability is at zero, and

the refractory period is absolute. Fatigue is asphyxiation; is the refractory period prolonged by a relative lack of oxygen.

The response to repeated stimuli depends on the irritability of the system at the moment of repetition. Oxidizable substances are being both broken down and built up during the refractory period. If the former is in the ascendance, irritability is diminishing, if the latter, increasing. The first result of each stimulus is a breaking down and therefore if the stimuli are repeated rapidly enough response is inhibited. If a sufficient interval intervenes the responses may be repeated, or even in heterobolic tissues (where the all or none law does not rule) may be summated.

Depression or retardation of vital processes can be most frequently explained on a basis of diminished oxidization. A depressant like a narcotic, for instance, diminishes oxidization and interferes with energy production. If O is transmitted to oxidizable substances by a carrier acting as a catalyzer the assumption that narcotics interfere with the carrier agrees with all the known facts concerning the action of narcotics. Other depressants (cold, fatigue, etc.,) likewise act through their interference with the processes of oxidation.

All the conclusions drawn in this work are well illustrated by numerous examples.

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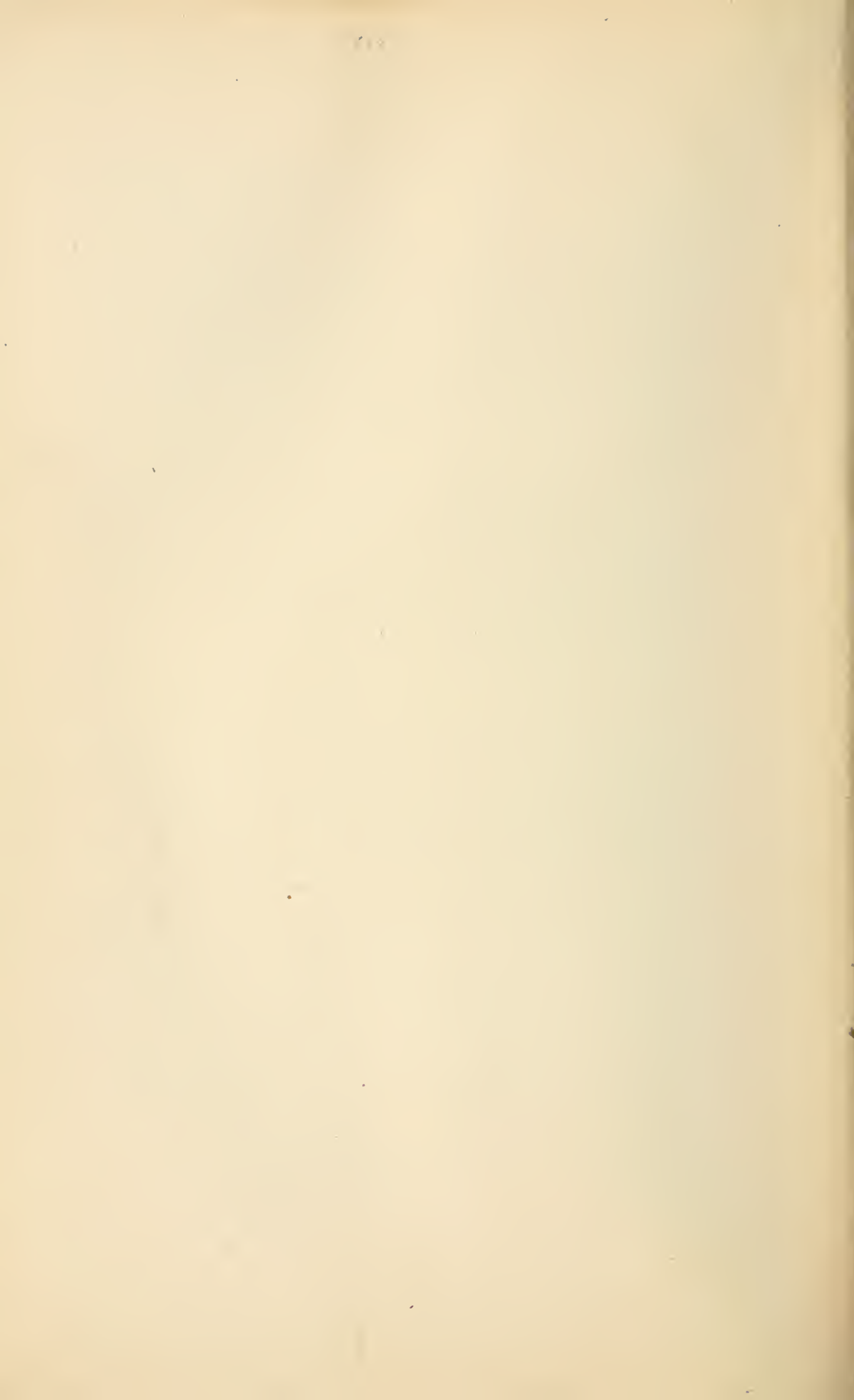


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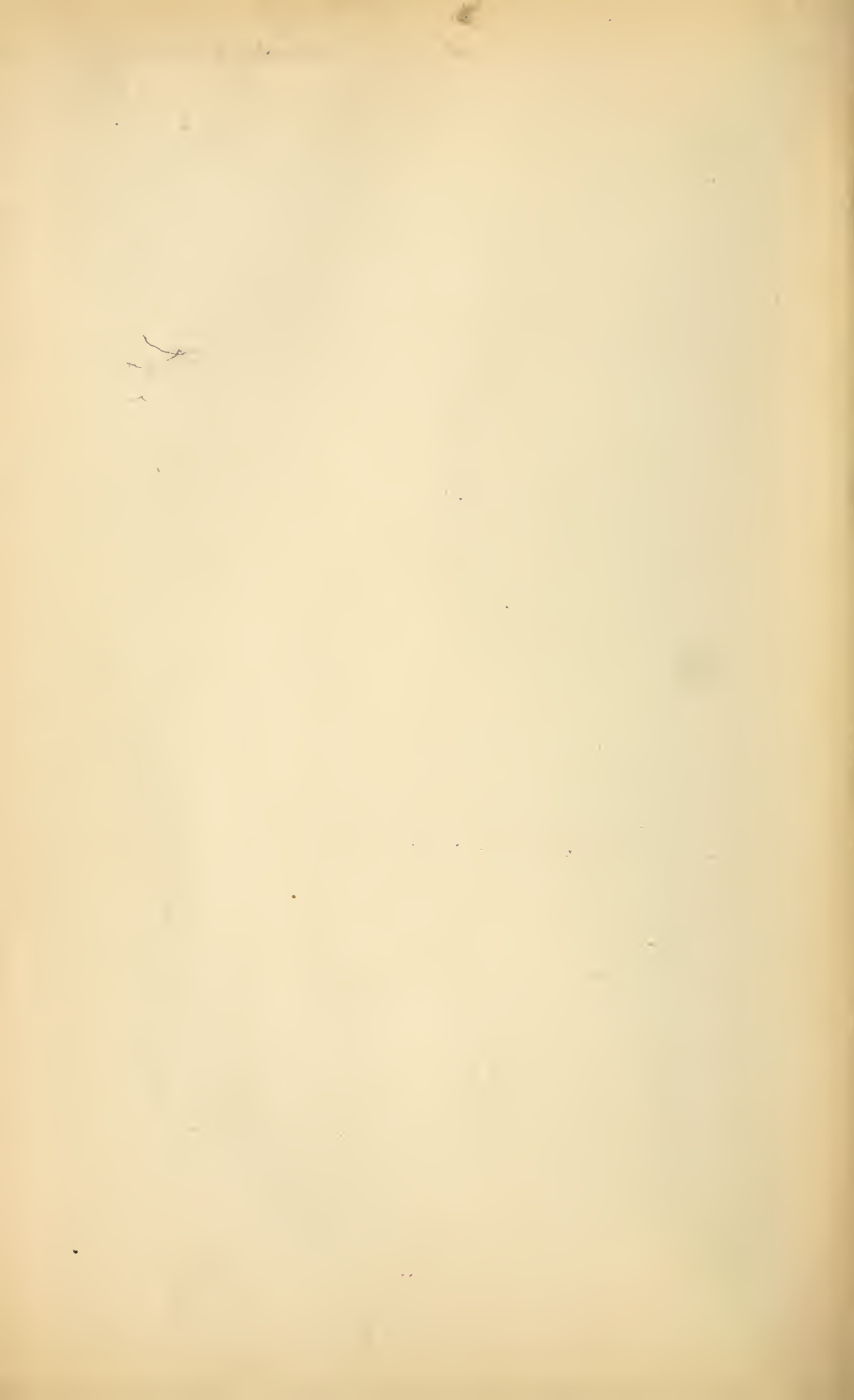
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